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CYSTINURIA IN SWEDEN

VII Clinical, histo-pathological, and medico-social aspects of the disease

BY

HARRY BOSTRÖM and LEIF HAMBRÆUS

STOCKHOLM 1964

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TO C. TH. MÖRNER

in appreciation of his pioneer research work on cystinuria

CONTENTS

INTRODUCTION

The important advances in biochemistry of the past two decades have stimulated increasing interest in congenital biochemical abnormalities. The introduction of chromatography and electrophoresis has opened up new possibilities in the study and diagnosis of these conditions.

Cystinuria is one of the diseases to which renewed attention has been paid. The condition is characterised by the excessive excretion of cystine, lysine, arginine, and ornithine in the urine and the tendency to form calculi in the urinary tract.

The main purpose of the present work was to ascertain the distribution of cystinuria in Sweden. The sparse and relatively static population of Sweden and the fact that vital statistics have long been available, make conditions favourable for investigating the familial distribution and frequency of hereditary disease. It was therefore hoped that the collection of the total known cases of cystinuria would permit a fair assessment of the possible need for centralising the care and prophylactic treatment of these patients.

Survey of the Literature

Foreign Literature on Cystinuria

Wollaston (1810) was the first to describe urinary calculi composed of cystine. He found in two bladder stones of unusual composition a substance which had previously been unknown and reacted with both acids and bases. Wollaston believed that the new substance was an oxide and named it cystic oxide because he had found it in a bladder stone. Berzelius (1830) demonstrated that this substance was not, in fact, an oxide and gave it the name cystine.

The first complete analysis of cystine was carried out by Thaulow (1838) who suggested the empirical formula $C_6H_{12}N_2S_2O_4$. Marcet (1817) was the first to observe that cystinuria was a familial disease. He reported on three cystinurics, two of whom were siblings. Examination of urine specimens from patients who formed such stones, showed typical cystine crystals in the sediment (Stromeyer 1824, Prout, 1823). This was believed to be due to excessive excretion of cystine. In quantitative studies on the urinary output of cystine Toel (1855) found that some cystinurics excreted between 1.33 g and 1.50 g cystine per day. These figures are in agreement with those reported by later workers (Stein, 1951, Harris & Warren, 1953, Dent *et al.*, 1954 *b*, Hambraeus, 1964 *a*).

Civiale (1838) was the first to review the cases of cystinuria published in the literature, which then numbered 19. In a later review of the pertinent literature Niemann (1876) found their number to have increased to 52. In the majority of these cases the condition was diagnosed by analysis of the available calculi but in a few cases it was recognised from the presence of cystine crystals in the urinary sediment. Niemann confirmed Marcet's and Civiale's observations that cystinuria was a familial disease and assumed that it was hereditary because one of his patients with this disease was only two years old. The literature on

cystinuria was further reviewed by Simon (1900) v Hofmann (1907) Link (191) Kretschmer (1916), Lewis (1931) Knox (1958) and others.

The incidence of cystinuria was at first believed to be very low Le 1 10 000–20 000 (Simon, 1900 Primavera quoted by Garrod, 1909 Sondern 1911) However at that time analysis of the calculi and examination of the urinary sediment were the only diagnostic methods available. It may therefore be assumed that many cases escaped attention. The use of the cyanide nitroprusside reaction (Brand *et al* 1930) enabled the diagnosis of excessive urinary excretion of cystine to be made with greater assurance. Lewis (1932) examined the cystine content of urine specimens from 10 000 healthy students at the university of Michigan and found the proportion of higher values than normal to be 1 600

Ackermann & Kutscher (1911) observed that cystinurics excreted large quantities not only of cy tine but also of lysine. In microbiological a says Yeh *et al* (1947) found that the urinary output of arginine was increased in addition to that of cystine and lysine in these patients Two years later Dent & Rose (1949) confirmed these observat ons by paper chromatography of urine pecimens from cystinurics. Stein (1951) made the same observation in ion exchange chrom tographic studies on the p ttern of u inary mino acid excretion in cyst n rics, and demonstrated that these pa tient lso excreted abnormally high quantities of orn thine

Cystinur a has long been regarded as an intermediate metabolic disease nd Sir Archib ld Garrod (1908, 1909 19 3) refer red to it a such in hi clas cal work Inborn Errors of Metabolism Ho ev r the observation that the plasma concentration of cystine was not increased in cystinuria (Brown & Lewis, 1937) argued against the view that the condition represented a metabolic disorder Freudenberg (1949) still believed that cystinuria and cystinosis were manifestations of the same inborn metabolic error In the same year Dent & Rose (1949) formulated the hypothesis that cystinuria was a renal disease and assumed that the immediate cause of the excessive excretion of cystine, lysine arginine, and ornithine in the urine was a defective capacity of the renal tubules to re-absorb these substances. From the observation that these amino acids resembled each other in possessing two positively charged amino-groups separated by a chain of four to six atoms, Dent & Rose (1951) suggested that there existed a common re-absorptive mechanism for these substances and that this mechanism was blocked in cystinuria. The disulphide between homocysteine and cysteine which Frimpter (1961) identified in the urine and demonstrated to be present in excessive quantities in cystinuria also appears to belong to this group of related diamines.

In the 1950's a series of papers on the aetiology treatment, and heredity of cystinuria appeared in the English medical literature (Arrow & Westall, 1958 Dent & Rose 1949 1951 Dent & Harris, 1951 Dent *et al* 1954 *a*, *b* Dent & Senior 1955 Fowler *et al* 195 Harris & Warren, 1953 1954 Harris & Robson 1955 Harris *et al* 1955 *a* *b* Robson & Rose 1957).

Harri and his co-workers (Harris & Warren, 1953 Harris *et al* 1955 *a*, *b*) ex mined urine specimens from the relatives of a series of cystinuric patients and demonstrated two genetical types of cystinuria, ... *ria* and *incomplete re*

cessive cystinuria. Patients with recessive cystinuria excrete abnormal amounts of cystine lysine, arginine, and ornithine (homozygous cystinuria) but the values of these substances in their relatives are within the normal range. Among the relatives of patients with incomplete recessive cystinuria individuals with normal values and individuals who excrete large quantities of cystine and lysine only are found. In the present paper the latter individuals are referred to as semi-cystinurics.

The observation that in cystinuria the re-absorption of the four amino acids concerned is deficient in the gut also (Milne *et al.* 1961, Asatoor *et al.*, 1962) argues against the view that it is a pure renal disease.

Swedish Literature on Cystinuria

The first case of cystinuria published in the Swedish medical literature was that reported by Enwall & Santesson (1874). The patient had formed a cystine stone which was removed from the "fossa navicularis" of the urethra by operation.

In the 1920's Mörner carried out extensive investigations on the distribution of cystinuria in Sweden and evolved a method for the concentration and demonstration of cystine crystals in the urine (Mörner 1922 *a*). Mörner succeeded in tracing 30 cases of cystinuria in Sweden and published the results of his investigations of these cases in a series of papers (Mörner 1922 *b* 1926 1932). He analysed the majority of the stones from these patients and estimated the cystine level in urine specimens from their relatives. He was also interested in the treatment of the disease and stressed the importance of decreasing the protein content of the diet of cystinurics (Mörner 1927).

In an extensive investigation of the solubility of cystine in urine Blix (1928) confirmed earlier observations on the solubility of this substance in water at different ion concentrations (Sano 1926) and studied the factors concerned in the higher solubility of cystine in urine as compared with its solubility in water. On the basis of his findings he assumed that urinary colloids interfere with the crystallisation of cystine.

Apart from Enwall & Santesson's case (1874) single cases of cystinuria were reported by Floderus (1910), von Holst (1912), Mörner (1921) Hammarsten (1931) Widmark & Hammarsten (1933), and Lauritzen (1957). In 1941 Renander published a monograph in which he reported on the results of his studies on the radio-opacity of cystine stones and reviewed the early literature on cystinuria.

In a screening examination of 7 700 school children, which included examination of the urinary excretion of cystine by the cyanide nitroprusside reaction, Boström & Tottie (1959) found the incidence of increased cystine excretion to be 1 : 500. The urine specimens in which the concentration of cystine was found to be raised were re-examined by paper chromatography and paper electrophoresis. From the results obtained the children were divided into two groups, those with homozygous cystinuria and those with semi-cystinuria, the proportion of the former being 1 : ...,600 and of the latter 1 : 560.

Preliminary reviews of the known cases of cystinuria in Sweden were published in 1959 (Boström, 1958/59) and in 1961 (Boström & Hambraeus, 1961).

MATERIAL

Compilation of Personal Data and Hospital Records of the Patients

The original case material on which the present work was based, comprised (1) the cases which Mörner had collected in the years from 192_ to 1936, and (2) those which Swedish urologists, surgeons and clinical chemists had encountered and had made accessible to us. To these were added the cases which were revealed by the above mentioned screening examination of 7 700 school children in Stockholm (Boström & Tottie 1959)

On the basis of Mörner's records or of the available hospital records we contacted the parish register offices in which the various cystinuri patients had been re gistered for census purposes when they had last been in contact with Mörner or under medical care and asked to send us a copy of their record on these patients and their relat ves. In this way information on the year of birth, birth place and the addres of the patients and their relati es at the time w s obtained A questionnaire inquiring about previous hospital or private med cal care was then sent to the patients and or their relat es thus traced, and the patient who were ali e were eventually interviewed personall

In the c ses n which the patient and his relatives h d died, information on the cause of death w obta ned from the parish re gister in th pace in which they had died

From 1956 to 1.86 data regarding the periods f r which t p tients over 16 years of age had e ed kness benef t nd hospital treatment, was obtained from the Sickness Benefit Societies in the different Counties of Sweden.

Case Histories

The case histories (see Appendix) were based on Mörner's records or the available hospital records of the patients Supplementary information was obtained by per sonal interview of the patients who were st'll alive either during their stay in a hospital in Stockholm or in the course of a field investigation carried out by one of us (H) some of the patients relatives be ng also inters ewed in the latter case.

Establishment of the Diagnosis

In the oldest cases in this series (Cases Nos. I 01 II 01 and XIX 03) in which the patient had died before 192_ (the year when Mörner began to collect the cases of cystinuria in Sweden) the diagnosis was made from the patient's past history and qualitati e and quantitati e analysis of the available urinary calculi

In the cases which Mörner collected in the years from 19 to 1935 the diagnosis rested on quantitati e analys of urinary pecimens and of the urinary calcul and microscopy of the urinary sediment.

Collection of Urine

N ght pecimens of urine from the pa tients and their relat ves were sent to the laboratory by post in 50 ml plastic bottles,

TABLE 1 *Specimens of Kidneys Obtained at Operation or Autopsy on 24 Cystinuric Patients*

Case No.	Sex	Age	Original examination macro-scopic	Original examination micro-scopic	Re-examination micro-scopic	Specimens obtained at: Biopsy	Partial nephrectomy	Nephrectomy	Autopsy
VIII. 01 03 01	Male	70	+	–	–	–	–	+	–
IX. 02 07	Female	71	–	+	+	–	–	–	+
XII. 01 07	Male	44	+	–	–	–	–	+	–
XVI. 01 06 05	Male	35	+	–	–	–	–	+	–
XVII. 01 03	Female	35	+	–	–	–	–	+	–
XIX. 02	Female	62	+	–	–	–	–	–	+
XIX. 03	Male	28	+	–	–	–	–	–	+
XXII. 01	Male	66	–	+	+	–	–	+	–
XXII. 06	Male	41	–	+	+	–	–	+	–
		56	–	+	+	+	–	–	–
XXIII. 01 04	Female	42	–	+	+	–	+	–	–
		44	–	+	+	–	+	–	–
		46	–	+	+	–	+	–	–
XXVI. 03 11	Male	31	–	+	+	–	–	–	+
XXIX. 01 05	Male	58	–	+	+	–	–		–
XXXIII. 06 03	Male	32	–	+	+	–	–	+	–
XXXV 01 02	Female	27	–	+	+	–	+	–	–
XLV 01 03	Male	26	–	+	+	–	+	–	–
		33	–	+	+	–	+	–	–
XLVI. 01	Male	27	–		+	–	+	–	–
XLVII. 01 02	Male	21	–	–	–	–	–	+	–
XLVIII. 01 03	Female	41	–	+	+	–	–	+	–
LI. 01 04 01	Male	48	–	+	+	+	–	–	–
LII. 03 06	Male	25	+	–	–	–	–	+	–
LIV 01 03	Male	15	+	–	–	–	–	+	–
LV 01 03	Female	52	–		+	–	–	+	–
LVI. 05	Female	52		+	+	–	–	–	+
LVI. 02 02	Female	26	–		+		–	+	–
Total			9	19	19	2	7	14	5

each containing a crystal of thymol to prevent contamination with bacteria, and were kept frozen at $-20°C$ until they were analysed.

Operation and Autopsy Specimens of Kidneys

Descriptions of the macroscopic and microscopic appearances of 28 operation or autopsy specimens of kidneys from 24 patients were available (Table 1).

A histological re-examination[1] of the available kidney specimens was performed in 15 of these 24 cases, twelve being operation specimens and the remaining three autopsy specimens. In 3 of the 15 cases unfixed operation or autopsy specimens of the

[1] Performed in collaboration with doctor A. Bergstrand.

kidney were obtained, and in the remaining 12 cases specimens embedded in paraffin wax were collected from different hospitals in Sweden. Several kidney operation specimens from the same patient were available for histological examination in 3 cases. Two of them (Cases Nos. XXIII. 01, 04 and XLV. 01, 03) underwent partial nephrectomy three times within four years and twice within seven years respectively, and in the third patient (Case No. XXII. 06) nephrectomy was carried out on one side and biopsy of the other kidney fifteen years later.

In 9 of the 24 cases the hospital records of the patients afforded information on the macroscopic appearance of the operation or autopsy specimens but not on their microscopic appearance, and no specimens were available for re-examination.

For microscopic re-examination the kidney specimens embedded in paraffin wax were sectioned and stained, using the following staining methods: van Gieson's, Haematoxylin-eosin, Ladewig's modification of Mallory's, and van Kossa's, the latter being used for the demonstration of calcium.

METHODS

Methods Used by Mörner

In the cases in which microscopy of the urinary sediment showed cystine crystals, Mörner carried out quantitative studies of the cystine in addition, using the following modification of Kondo's (1915) method (Mörner 1922 *a*)

(1) The spontaneously deposited urinary sediment is suspended in 15 ml of a 0.1% solution of acetic acid and filtered. The residue is washed in 10 ml of a 0.1% solution of acetic acid.

(2) The rest of the urine is filtered, acidified, and freed from albumen if present. 10 ml of a 10% solution of ammonia is then added to 200 ml of the urine. The mixture is stored in a closed vessel for two days and then filtered to free it from calcium phosphate, magnesium phosphate calcium oxide, and ammonium urate 4.5 ml of concentrated acetic acid is added to 157.5 ml of the filtrate (which corresponds to 150 ml of urine) and the solution is evaporated to dryness. The resultant residue is dissolved in 15 ml of a 0.1% solution of acetic acid (to dissolve alkaline salts) and filtered. The residue is then washed in 10 ml of a 0.1% solution of acetic acid.

The residue left on the filter after the above two procedures is treated as follows: The residue is washed into a beaker with 10 ml of water. It is then washed in 10 ml of diluted hydrochloric acid (1 part 25% hydrochloric acid and parts water). The mixture is filtered after 24 hours (urates being thereby separated) and the residue is washed three times with 1 ml of water. The filtrate together with the washing fluid is evaporated to dryness (cystine is thereby deposited in the form of hydrochloride), redissolved in 5 ml of a 5% solution of ammonia, allowed to stand for 24 hours and filtered. The residue is washed three times with 1 ml of a 5% solution of ammonia, being thereby freed from possible remaining phosphates and calcium oxide. The filtrate together with the washing fluid is again evaporated to dryness. Cystine appears then in the form of crystals, together with impurities such as ammonium chloride and sodium chloride if present.

The crystals are washed with 5 ml of a 0.1% solution of acetic acid and filtered. The residue is washed twice with 1 ml of a 0.1% solution of acetic acid, twice with 1 ml of absolute alcohol, and twice with 1 ml of ether. The crystals are then dried and weighed.

According to Mörner (1922 *a*) polarometric examination of cystine isolated by the above method showed it to be free from impurities, and Kondo (1915) and Mörner (1922 *a*) assessed the recovery of the method to be 80%. Mörner examined the cystine content of urine specimens from 0 cystinuric patients by this method and found that it ranged between 0.025 g and 1.15 g per litre of urine.

For analytical study of the urinary calculi Mörner used the following method (Mörner 1922 *b* Case No. VIII):

Fragments of the calculi are dissolved in a 5% solution of ammonia. The solution is allowed to evaporate in the air and, following this, colourless crystals appear. These are dissolved in diluted hydrochloric acid to which sodium acetate has been added (to obtain a negative Congo red reaction). Following this procedure hexagonal crystals appear which are weighed.

Mörner also determined the ash content of the calculi.

TABLE 3 *Familial Distribution of Homozygous Cystinuria*

No. of families	No. of cases per family	Total no. of cases
38	1	38
9	2	18
8	3	24
2	4	8
2	5	10
Total 59		98

Primary Secondary and Screening Cases of Cystinuria

The cases in the present series were divided into the following three groups (Table 4)

Group 1 Primarily diagnosed cases (primary cases) This group comprised 56 patients (39 male and 17 female) in whom the diagnosis of cystinuria was made when they developed urinary tract symptoms, and was based on chemical analysis of stones removed by operation or on urinalysis.

Group 2 Secondarily diagnosed cases (secondary cases) This group consisted of 38 individuals with cystinuria (17 male and 21 female) who were relatives of the above 56 patients and from whom specimens of urine were collected and cystinuria was recognised in conjunction with the investigation of the familial occurrence of the disease. Mörner traced 10 and the present authors 28 secondary cases.

Group 3 Screening cases This group comprised 4 cases (1 boy and 3 girls) in whom the diagnosis was made at the screening examination of healthy school children mentioned above (Boström & Tottie, 1959). It is interesting to note that neither the children nor their relatives had ever had urinary tract symptoms.

Sex and Age Distribution

Of the 98 patients with homozygous cystinuria 25 (17 male and 8 female) had died before 196_. Of the 72 patients still alive in 1962 39 were male and 33 female (Table 4). One male patient (Case No V 02) could only be followed up until 1971 when he was 39 years old, and was therefore not included in Tables 4 5 and _1–_5

Table 5 shows the age distribution of the 7_ living cystinurics as determined in 196_. It is seen that the majority were between the ages of ?0 and 60 years.

TABLE 4 *Distribution of Primary Secondary and Screening Cases in the Total Case Material*

	Primary cases*			Secondary cases			Screening cases			Total		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Alive	23	13	36	15	17	32	1	3	4	39	33	72
Dead	15	4	19	2	4	6	—	—	—	17	8	25
Total	38	17	55	17	1	38	1	3	4	56	41	97

*Case No. V 02 not included

Table 5 *Age Distribution of 72 Living Cystinuric Patients*

Age (years)	No. of cystinurics		
	Male	Female	Total
0–10	1	—	1
11–20	3	5	8
21–30	4	5	9
31–40	15	7	22
41–50	8	2	10
51–60	3	1	15
61–70	5	1	6
> 70	—	1	1
Total	39	33	72

Case No. V 02 not included.

Table 6 *Distribution of the Known Cases of Cystinuria Diagnosed in the Different 10-Year Periods from 1900–1962*

Time	No. of cystinurics		
	Male	Female	Total
Before 1900	1	—	1
1901–10	4	—	4
1911–20	3	1	4
1921–30	11	10	21
1931–40	2	2	4
1941–50	3	2	5
1951–60	31	22	53
Since 1960	2	4	6
Total	57	41	98

Observation Time

Table 6 shows the number of cases diagnosed in the different 10-year periods from 1900 to 1962. It is seen that the majority were diagnosed between 1921 and 1930 (21%) and after 1950 (60%).

Table 7 shows the number of patients born within the different 10-year periods. Table 8 shows the correlation between the number of patients and the length of the observation time. It is seen from Table 7 that 25 patients were born before 1900. Table 8 shows that the observation time of 50 patients with homozygous cystinuria was more than 40 years.

Table 7 *Number of Cystinuric Patients Born Within the Different 10-Year Periods Since 1850*

Year of birth	No. of cystinurics		
	Male	Female	Total
Before 1850	2	—	2
1851–60	3	—	3
1861–70	—	1	1
1871–80	2	2	4
1881–90	3	4	7
1891–1900	6	2	8
1901–10	5	11	16
1911–20	10	4	14
1921–30	16	7	23
1931–40	6	4	10
1941–50	3	5	8
1951–60	—	1	1
After 1960	1	—	1
Total	57	41	98

Table 8 *Correlation Between Number of Cystinuric Patients and Length of Observation Time*

Observation time	No. of cystinurics		
	Male	Female	Total
> 80 years	1	1	
> 70 years	3	5	8
> 60 years	12	8	20
> 50 years	18	22	40
40 years	27	23	50
30 years	47	31	78
> 20 years	54	36	90
> 10 years	56	41	97
< 10 years	57	41	98

TABLE 9 *Diagnostic Methods Used*

			Urinalysis					Stone analysis	
Case No.	Family history	Patient's previous history	Urinary sediment[a]	Mörner	Brand	Paper chromatography	Paper electrophoresis	Chemical methods	Roentgen crystallography
I. 01		+	−					+	
II. 01	−	+						+	
III. 01 01	−	−	+	−				+	+
IV. 05	−	+	+	−					
V. 02	−	+		+					+
VI. 01 02	+	+	+	+					+
VI. 01 04	+	−		+					
VI. 01 07	+	+		+					
VII. 01	−	+		+				+	
VIII. 01 03 01	−	+		+	+	−	−	−	
IX. 02.07	+	+	+	+				+	+
IX. 01 01-06	+	+			−	+	+		
X. 06		+		+				−	+
XI. 01	?	+		+				+	+
XII. 01-02	−	+		+		+			
XII. 01 07	+	+		+	+	−	+	+	
XIII. 01-01-01	−	+		+	+	+	+		
XIV. 01-01	+	+						−	+
XIV. 01-02	+	+		+	+	+			
XV. 01 01 01	−	+	+	−				+	+
XVI. 01 06 02	+	+	+	−	+	+			+
XVI. 01 06 05	−	+			+	+		+	
XVI. 01 06-06	+	−		−		+	+		
XVII. 01 01	+	−			−	+	+		
XVII. 01 03	+	−			+	+	+	−	
XVII. 01-06	+				−	+	+		+
XVIII. 02		+							
XIX. 07	+								
XIX. 03	+								
XIX. 05	+	+							
XIX. 04 01-01						−	+		
XX. 01 01	−	−				+	+		
XX. 01-04	−						+	+	+
XX. 01-08	−						+		
XXI. 05								+	
XXII. 01	+				+		+		
XXII. 06					+				+
XXIII. 01 02							−		
XXIII. 01 04						−		+	
XXIII. 01 07						−			

[a] Examined during hospital treatment of patient.

Table 9 (*continued*)

Case No.	Family history	Patient previous history	Urinalysis					Stone analysis	
			Urinary sediment[a]	Mörner	Brand	Paper chromatography	Paper electrophoresis	Chemical methods	Roentgen crystallography
XXIV· 02 01	−	+			+	+	+	+	+
XXV 01 03		+			+	+	+	+	+
XXVI. 03 05	+	+							
XXVI. 03 07	+	+	+		+	+	+		
XXVI. 03 09	+	+			+	+	+		
XXVI. 03 11	+	+	+		+	+	+		+
XXVI. 03 12		+			+	+	+		
XXVII. 01 04 01		+	+		+	+	+		+
XXVIII. 01 02 01					+	+	+		
XXIX. 01 04	+	+						+	
XXIX. 01 05	+	+			+	+	+	+	+
XXX. 01-05		+			+	+	+	+	+
XXXI. 01	+	+			+	+	+		
XXXI. 03	+	+	+		+	+	+	+	
XXXI. 03 01	+	+	+		+	+	+		
XXXII. 01 03	+	+	+		+	+	+		
XXXII. 01 04	+	+	+		+	+	+	+	+
XXXIII. 06-03		+	+		+	+	+	+	+
XXXIV 02	+	+			+	+	+	+	+
XXXIV 04	+	+			+	+	+	+	+
XXXIV 06	+	+			+	+	+		
XXXV 01 02		+			+	+	+	+	+
XXXVI. 01 01		+			+	+	+	+	
XXXVII. 01 01		+	+		+	+	+	+	
XXXVIII. 02 04		+			+	+	+	+	+
XXXIX. 01 02 02		+			+	+	+		
XL. 01 01 01					+	+	+		
XL. 01 01 02					+	+	+		
XLI. 01 01 01		+			+	+	+		
XLII. 01 04		+			+	+	+	+	+
XLIII. 01 03		+	+		+	+	+		+
XLIV 03 02		+			+	+	+	+	+
XLV 01 03		+			+	+	+	+	
XLVI 01		+			+	+	+	+	
XLVII. 01 01	+				+	+	+		
XLVII. 01 02		+			+	+	+		
XLVII. 01 04	+	+			+	+	+	+	
XLVIII. 01 03		+			+	+	+	+	
XLVIII. 01 04		+			+	+	+		
XLVIII. 01 07	−				+	+			
XLVIII 01 09	+				+	+	+		
XLIX. 02 02		+			+	+	+	+	+

Table 9 (*continued*)

Case No.	Family history	Patient's previous history	Urinary sediment	Mörner	Brand	Paper chromatography	Paper electrophoresis	Chemical methods	Roentgen crystallography
			Urinalysis					Stone analysis	
L. 01 02						+	+	+	+
L. 01 03						+	+		
LI. 01 04 01		+				+	+		+
LII 03 06						+	+	+	+
LII 03 09	+					+	+		
LIII. 01 03		+				+	+	+	
LIV 01 03						+	+	+	
LV 01 03	–	+				+	+	+	
LVI 05						+	+		
LVI 09	+					+	+		
LVI 07 02	+					+	+	+	
LVI. 02 03	+	–				+	+		
LVI. 02 04						+	+		
LVII 01						+	+	+	
LVIII. 01 03 01	–	+				+	+		+
LIX. 06	?	+			–	+	+	+	
Total: 98 Cases		87 + 11 –	26	21	77	77	77	53	34

Examined during hospital treatment of patient.

Criteria of Cystinuria

The methods used for the diagnosis of cystinuria in each case are given in Table 9

In 6 cases routine urinalysis, carried out when the patient was in hospital, showed cystine crystals in the sediment.

Using the method described earlier Mörner diagnosed excessive excretion of cystine in the urine in 21 cases.

In 77 cases, including 8 of the above 21 cases examined by Mörner Brand's test, two-dimensional paper chromatography and paper electrophoresis showed the urinary excretion of cystine, lysine, arginine, and ornithine to be increased.

In 59 cases the diagnosis of cystinuria was verified by stone analysis which showed that the calculi were composed predominantly of cystine.

In 28 cases chemical analysis of the urinary calculi was combined with roentgen crystallographic studies, examining one (10 cases) or more calculi (18 cases). Roentgen crystallography alone was carried out in 6 cases.

In 55 cases the family histories revealed that cystinuria was familial. In 5 cases this could not be conclusively proved.

In 87 cases there was a previous history of urinary symptoms and in 11 cases the patients had never had any urinary symptoms. The average age of these 11 patients

in 196_ was 23.3 years 3 of them were children born in 1950 or 1951 and belonged to the group screening cases.

Summarising it may be said

(1) In 54 cases stone analysis revealed that the urinary calculi were composed predominantly of cystine, and urinalysis demonstrated an abnormal excretion of cystine. In 44 of these cases urinalysis was performed by Brand's test, two-dimensional paper chromatography and paper electrophoresis. In the remaining 10 cases it was carried out by Mörner's method, which permitted a quantitative estimate of the excretion of cystine to be made.

(2) In 36 cases the diagnosis was made from urinalysis alone in 33 of these Brand's test, two-dimensional paper chromatography and paper electrophoresis were used, and in the remaining 3 cases urinalysis was carried out by Mörner' method.

(3) In 3 cases stone analysis alone was performed and was positive for cystine. In another 2 cases microscopy of the urinary sediment was carried out in addition to stone analysis and revealed cystine crystals.

(4) In 3 cases the diagnosis was made after the patient's death from his history (severe urinary tract symptoms similar in type to those characterising cystinuria) and from information on the occurrence of cystinuria in the patient's family (the patient's siblings having had the disease).

Symptoms and Signs of Cystinuria[1]

The case histories (see Appendix) showed that the principal clinical features of cystinuria were urinary tract symptoms, the clinical course of the disease varying greatly from case to case.

Of the 98 patients 87 had urinary tract symptoms the remaining 11 patients never had any such symptoms. Six patients in the former group (Cases Nos. VI. 01 07 XVII. 01 01 XXIII. 01 02, XXVI. 03 09 XXXIX. 01 02 02, LII. 03 08) all women, had had cystitis on one or more occasions, this being their only symptom. As their history regarding the time of onset of the disease and the severity of the symptoms was unreliable, they were not included in Tables 10, 11 and 20.

TABLE 10 *Initial Symptoms in 81 Cystinuric Patients*

Initial symptom	No. of cystinurics		
	Male	Female	Total
Renal colic or renal ache	37	20	57
Infection (cystitis, pyelocystitis, pyelitis)	11	10	21
Retention of urine	1	—	1
Painless haematuria or passage of stone	2	—	2
Total	51	30	81

In Table 10 the initial symptoms in the above 81 patients, are given. In the majority (57 patients, 37 male and 20 female) renal colic (the pain radiating to the groin or being experienced as a gnawing pain in the renal region) was the initial symptom, there being no demonstrable urinary infection.

In 21 cases the first symptoms were referable to a urinary tract infection, *i.e.* there was frequent and painful micturition in 13 cases (9 males and 4 females) which suggested cystitis, and pain in both renal regions associated with pyrexia and bacteriuria, findings which suggested pyelitis, in 8 cases (? males and 6 females). In 1 male patient (Case No VIII 01 03 01) retention of urine was the first symptom, 1 male patient (Case No. XIX. 03) had haematuria

[1] The 4 screening cases—the oldest of these patients being 12 years in 196_—were not included in Tables 10 11 13 15 19 and 20, because their number was too small and the observation time too short.

TABLE 11 *Age of 98 Cystinuric Patients at Onset of Symptoms*

Age (years)	Primary cases			Secondary cases			Total
	Male	Female	Total	Male	Female	Total	
Before 10	6	—	6	—	—	—	6
10–19	9	6	15	1	—	1	16
20–29	16	4	20	7	7	14	34
30–39	3	4	7	4	3	7	14
40–49	2	2	4	—	1	1	5
50–59		1	3	—	—	—	3
After 60	1	—	1	1	—	1	
No symptoms[a]	—	—	—	4	4	8	8

[a] Average age in 1962: 27 6 years.

and passed gravel at 22 years of age without any premonitory symptoms, and 1 male patient (Case No. LVIII. 01 03 01) had *passed calculi spontaneously at the age of 8* months without having had any previous urinary symptoms.

Table 11 shows that the first symptoms generally appeared before the age of 40 The average age of the 8 patients who were alive in 196 and who had never had any urinary tract symptoms, was 27 6 years.

In — patients (16 male and 6 female) the first symptoms appeared before the age of 20. Of the total number of male patients (56) 6 had their first urinary tract symptoms before the age of ten, the 3 women in the series had never had any symptoms before this age

Table 11 shows that the first symptoms appeared somewhat earlier in the primary cases than in the secondary cases. Of the 22 cases in which the first symptoms occurred before the age of twenty .1 were primary cases and 1 was a secondary case

Fig. 1 shows a diagram of the percentage distribution of the patients according to the age when the first symptoms appeared. It is seen that 91 % had had urinary symptoms before the age of sixty

Table 12 shows that of the 98 cystinuric patients 69 (45 male and 24 female) had one or more attacks of renal colic. Fifty seven patients (39 male and 18 female) passed calculi or gravel spontaneously Pain in the renal region was an additional symptom in 44 patients (29 male and 15 female)

Sixteen of the 98 patients (13 male and 3 female) occasionally had anuria. In 9 cases (7 males and 2 females) the patient had only one functioning kidney the cause of the anuria being a ureteric calculus. In one of the 7 male patients (Case No XLVII. 01 04) there was agenesis of the



Fig. 1

TABLE 12. *Symptoms Experienced by the Cystinuric Patients*

Urinary tract symptoms	No. of cystinurics		
	Male	Female	Total
Renal colic	45	24	69
Renal ache	29	15	44
Passage of stone or gravel	39	18	57
Frequency of micturition	25	19	44
Anuria	13	3	16
Dysuria	20	17	37

TABLE 13 *Incidence of Different Symptoms*

Urinary tract symptoms	40 years observation time %	Total case material* %
Renal colic	84	73
Renal ache	46	47
Passage of stone or gravel	68	61
Frequency of micturition	46	46
Anuria	18	17
Dysuria	36	38

* Screening cases not included.

other kidney and in a further male patient (Case No. XXXVIII 07 04) the other kidney had ceased to function when he was a child. In the other 7 cases of solitary functioning kidney the other kidney had previously been removed.

In the remaining 7 of the 16 patients with anuria the condition was due to a calculus obstructing the urethra in 4 male patients; in another male patient (Case No XXVI. 03 05) there was calculous obstruction of *both* ureters in a further male patient (Case No. XV 01 01 01) and 1 female patient (Case No. XXI 05) the cause of the anuria could not be demonstrated.

Frequency of micturition and painful micturition occurred in 44 cases (25 males and 19 females) and 37 cases (20 males and 17 females) respectively

The percentage incidence of the above symptoms in the total case material and in the cases in which the observation time was 40 years, is shown in Table 13

The clinical and laboratory findings in the patients in this series are given in Table 14 The Table was based on the information obtained from the hospital records of the patients.

In 1 male patient a vesical calculus was palpable and in 28 patients (16 male and 12 female) the kidneys were tender on palpation.

In 36 patients (28 male and 8 female) there was macroscopic haematuria and in 37 patients (21 male and 16 female) microscopic haematuria only

Albuminuria and bacteriuria were noted on one or more occasions in 39 patients (21 male and 18 female) and 45 patients (28 male and 17 female) respectively

In 38 patients (25 male and 13 female) the serum N.P.N and/or the serum creati-

TABLE 14 *Clinical and Laboratory Findings in the Cystinuric Patients*

Findings	No of cystinurics		
	Male	Female	Total
Palpable vesical calculus	1	—	1
Renal tenderness	16	12	28
Pyrexia	20	16	36
Macroscopic haematuria	28	8	36
Microscopic haematuria (only)	21	16	37
Albuminuria	21	18	39
Bacteriuria	28	17	45
Elevated serum N P N or serum creatinine	25	13	38

TABLE 15 *Incidence of Different Clinical and Laboratory Findings*

Findings	40 years observation time %	Total case material* %
Palpable vesical calculus	2	1
Renal tenderness	28	30
Pyrexia	39	38
Macroscopic haematuria	35	38
Microscopic haematuria (only)	42	39
Albuminuria	42	4.
Bacteriuria	46	48
Elevated serum N P N. or serum creatinine	42	40

* Screening cases not included.

nine were occasionally or permanently raised, being more than 40 mg% and more than 1 0 mg% respectively In 36 patients (20 male and 16 female) there was pyrexia.

Table 15 shows the percentage incidence of the above clinical and laboratory findings in the total case material and in the cases in which the observation time was 40 years or more.

Data on .59 blood pressure readings were available in 66 patients (Table 16) In 15 of these information on the blood pressure readings on one occasion only was available Forty-one (6.%) patients (27 male and 14 female) had a blood pressure below 160/100 mmHg, in 8 (12%) patients (5 male and 3 female) it was on one or more occasions between 160/100 mmHg and 180/110 mmHg and in 17 (26%) patients (8 male and 9 female) it was more than 180/100 mmHg. In Table 17 the last blood pressure readings in each of the 66 cases are given and compared with the patients ages. It is seen that 13 (40 6%) of the 32 patients above 40 years of age had a blood pressure of more than 180/100 mmHg and that only 2 patients younger than 40 years had a blood pressure above 180/100 mmHg.

As a rule the symptoms and signs described were experienced bilaterally (Table 18) Of the 98 patients 14 experienced symptoms on one side only whilst 50 patients had symptoms on both sides. In 14 cases no information regarding laterality was available. In 9 cases (3 males and 6 females) the symptoms were those of cystitis only In 11 patients (5 male and 6 female) cystinuria was asymptomatic. Bilateral symptoms were commonest also in the cases in which the observation time was 40 years or more.

Bilateral symptoms appeared to be more common in men than women (Table 18 *b*) and to be more frequent in the primary cases than in the secondary cases (Table 18 *c*)

TABLE 16. *Blood Pressure Readings in 66 Cystinuric Patients*

Sex	Total cases	Blood pressure readings (mmHg) <160/100	160–180/100–110	>180/100
Male	40	27	5	8
Female	26	14	3	9
Total	66	41	8	17

TABLE 17 *Comparison of Blood Pressure Readings with the Age of 66 Cystinuric Patients*

Sex	Age at last blood pressure measurement (years)	Blood pressure readings (mmHg) <160/100	160–180/100–110	>180/100	Total
Male	<20	1	—	—	1
	20–29	11	—	—	11
	30–39	10	—	1	11
	40–49	4	—	3	7
	50–59	3	—		5
	>60	3	—	2	5
Female	<20	1	—	—	1
	20–29	5	—	1	6
	30–39	3	1	—	4
	40–49	—	1		3
	50–59	8	—	1	9
	>60	—	—	3	3
Total		49	[illegible]	15	66

The sites of the calculi are given in Table 19 which shows that in 49% of the cases the calculus was lodged in the right and in 36% in the left renal pelvis, the corresponding percentages for the ureteric calculi being 18% and 21% respectively. In 60% of the cases the patient passed stones spontaneously.

As far as the percentage distribution of the sites of the urinary calculi was concerned there was no difference between the cases with a follow-up of 40 years and the total case material. The frequency of lithiasis was on the average lower in women than in men, irrespective of the site of the calculi.

TABLE 18 *Location of Urinary Tract Symptoms in Different Groups of Cases*

	Urinary tract symptoms absent	Upper urinary tract symptoms: Unilateral	Bilateral	No information available regarding side	Bladder symptoms only
a) Total case material	11	14	50	14	9
a) 40 years observation time	1	8	27	10	4
b) Male	5	7	34	8	3
b) Female	6	7	16	6	6
c) Primary cases	—	9	37	8	3
c) Secondary cases	8	6	13	6	5
c) Screening cases	3	—	—	—	1

TABLE 19 *Site of Urinary Calculus*

Site of calculus	40 years' observation time		Total case material[a]	
	No. of cases	%	No. of cases	%
Calculus in left renal pelvis	17	34	34	36
Calculus in right renal pelvis	22	44	46	49
Left ureteric calculus	*8*	*16*	20	21
Right ureteric calculus	9	18	17	18
Vesical calculus	9	18	14	15
Urethral calculus	3	6	8	9
Calculus passed spontaneously	34	68	57	60

[a] Screening cases not included

Recurrence of Urinary Tract Symptoms

Table 20 shows that the number of episodes of urinary tract symptoms varied greatly. Of the 89 patients in whom reliable information on this point was available only 8 patients (9 %) had never had any urinary tract symptoms. The cases observed for 40 years or longer differed in this respect, only 2 % of the patients never having had any such symptoms. There was hardly any difference between the sexes with respect to absence of urinary tract symptoms, *i.e.* 7 % of the male patients had never had any symptoms as compared to 12 % of the female patients (Table 20 *b*).

In 6–7 % of the cases there was a single episode of urinary tract symptoms. There was no noteworthy difference between the total cases and those followed up for 40 years in this respect (Table 20 *a*).

In 84 % of the total case material and 93 % of the cases in which the follow-up was more than 40 years, urinary tract symp-

TABLE 20 *Number of Episodes of Symptoms in Different Groups of Cases*

Number of episodes of urinary tract symptoms	0		1		2 or more		
	No. of cases	%	No. of cases	%	No. of cases	%	Total No. of cases
a) Total case material	8	9	6	7	75	84	89
a) 40 years observation time	1	2	3	6	50	93	54
b) Male	4	7	5	9	47	84	56
b) Female	4	12	1	3	28	85	33
c) Primary cases	—	—	3	5	53	95	56
c) Secondary cases	8	24	3	9	22	67	33

toms recurred. Table 20 *b* shows that the recurrence percentage for the male patients was 84% and for the female patients 85%. The recurrence percentage was higher in the primary cases than in the secondary cases, being 95% and 67% respectively. However it should be borne in mind that in 24% of the secondary cases cystinuria had been asymptomatic. The above recurrence percentages refer to the total case material. If the corresponding percentages for the patients followed up for more than 40 years are taken into consideration it will be found that the difference between primary cases and secondary cases, and between male and female patients with respect to the recurrence percentages is much smaller

TABLE 21 *Number of Operations Performed on the 97 Cystinuric Patients in This Series*[a]

No. of operations	No. of cases		Total cases
	Males	Females	
0	17	22	39
1	11	11	22
2	8	4	12
3	7	1	8
4	4	0	4
5	3	1	4
6	3	1	4
7	2	1	3
8	1	—	1
Total	56	41	97

[a] Case No V 02 not included.

Operations by which Urinary Calculi Were Removed

Table 21 shows the total number of operations by which urinary calculi were removed. It is seen that 39 patients (40%), *i e* 30% of the total number of male patients and 54% of the total number of female patients, were not operated upon 22 patients (23%) were operated upon once, and 36 patients (37%) underwent between two and eight operations.

Table 22 shows the types of operation performed. It is seen that pyelolithotomy was the commonest operation, the next in order of frequency being ureterolithotomy Cystolithotomy was performed nineteen times *i.e.* once upon 1 female patient and eighteen times upon 1 male patients. In 7 male patients nine urethral calculi were extracted. Fourteen patients (10 male and 4 female) underwent nephrectomy

The type of the initial operation performed upon patients who underwent several operations, is shown in Table 23 It is seen that pyelolithotomy was the first operation in the majority of these cases.

Table 24 shows the age of the patients at the time of the initial operation. In 8 cases the diagnosis of cystinuria was made prior to the first operation and in 28 cases after it. In the remaining 22 cases the condition was not recognised until the second to sixth operation had been performed (Table 25)

Nephrectomy

Fourteen patients underwent nephrectomy Seven of these patients had had no previous operation for urinary calculus (Table 26) The interval between the onset of urinary tract symptoms and nephrectomy varied between six months and thirty-six years, the average interval being sixteen years (Table 26)

The indication for nephrectomy was poor renal function or hypertensive symptoms in

TABLE 22. *The Different Operative Procedures Carried Out on the 58 Surgically Treated Cases*

Type of operation	No of cystinurics*					
	Males		Females		Total	
	No of cases	No. of operations	No. of cases	No. of operations	No of cases	No. of operations
Left nephrectomy	4	4	1	1	5	5
Right nephrectomy	6	6	3	3	9	9
Left pyelolithotomy	13	18	6	9	19	27
Right pyelolithotomy	20	27	12	17	32	44
Left pyelolithotomy + ureterolithotomy	1	1	1	1	2	2
Right pyelolithotomy + ureterolithotomy	4	4	1	1	5	5
Left ureterolithotomy	9	13	4	5	13	18
Right ureterolithotomy	9	11	2	2	11	13
Endoscopic removal of ureteric calculus	2	2	—	—	2	2
Ureterolithotomy + cystolithotomy	1	1	—	—	1	1
Suprapubic cystolithotomy	12	18	1	1	13	19
Perurethral extraction of urethral calculus	7	9	—	—	7	9

Case No. V 02 not included

TABLE 23 *Type of Initial Operation*

Type of operation	No. of cystinurics*		
	Males	Females	Total cases
Nephrectomy	4	3	7
Pyelolithotomy	10	11	21
Pyelolithotomy ureterolithotomy	2	—	2
Ureterolithotomy	7	4	11
Endoscopic removal of ureteric calculus	2	—	2
Suprapubic cystolithotomy	9	1	10
Perurethral extraction of urethral calculus	5	—	5
Total	39	19	58

Case No. V 02 not included.

6 cases, and severe unilateral renal pain in 4 cases. Two of the latter patients later developed pain on the contralateral side also. In 4 cases a coral stone was removed by nephrectomy

Macroscopic or microscopic examination of the nephrectomy specimens revealed changes of varying degree of severity in the renal parenchyma which were consistent with chronic pyelonephritis in all cases. (For a more detailed description of the histological findings see p 31.)

Of the 14 patients who underwent nephrectomy urinary tract symptoms recurred after a longer or shorter interval in 8 In each of these 8 cases at least one more additional operation on the urinary

TABLE 24 *Age at Time of Initial Operation*[a]

Age (years)		<10	10–19	20–29	30–39	40–49	50–59	>60
a)	Males	5	7	14	7	3	1	2
	Females	—	2	6	4	5	2	—
	Total	5	9	20	11	8	3	2
b)	Primary cases	5	9	16	8	5	2	1
	Secondary cases	—	—	4	3	3	1	1

[a] Case No. V 02 not included.

tract was performed. The age of these patients at nephrectomy varied between 15 years and 52 years (average age 32.6 years) and the post-operative observation time be tween 5 years and 25 years (average post operative observation time 16.1 years). The age at nephrectomy of the remaining 6 patients who remained symptom-free post operatively varied between 21 years and 66 years (average age 41 7 years) and the post operative observation time between 1 year and 24 years (average post-operative observation time 10.5 years)

Of the 14 patients who underwent nephrectomy blood pressure readings before and after the operation were given in the hospital records of 9 Five of these patients had a blood pressure below 160/100 mmHg before and four of them after nephrectomy· the fifth patient had a blood pressure of 180/100 mmHg twenty four years after the operation. The 4 remaining patients had a blood pressure above 160/100 mmHg prior to nephrectomy In two of these patients it decreased after the operation and in the other .. patients the blood pressure readings were 195/105 mmHg and 210/140 mmHg respectively fifteen years and seventeen years after nephrectomy

Results of Stone Analysis

According to the hospital records 130 calculi from 59 patients were composed predominantly of cystine (Table 27) Ten

TABLE 25 *Number of Operations Performed before the Diagnosis of Cystinuria Was Established*[a]

No. of operations		0	1	2	3	4	5	6	Total cases
a)	Males	2	20	7	4	3	1	2	39
	Females	6	8	3		—	—	—	19
	Total	8	28	10	6	3	1	2	58
b)	Primary cases	6	21	8	6	2	1	2	46
	Secondary cases	2	7	2	—	1	—	—	12

[a] Case No. V 02 not included

TABLE 26. *Nephrectomy Cases*

Case No.	Sex	Age at nephrectomy (years)	Interval between appearance of symptoms and nephrectomy (years)	No. of operations for urinary calculi performed before nephrectomy	Recurrence of urinary symptoms after nephrectomy	Observation time after nephrectomy (years)
VIII. 01 03-01	Male	20	17	2	+	25
XII 01 07	Male	44	18	3	−	4
XVI. 01-06 05	Male	35	17	2	+	4
XVII. 01 03	Female	35	23	1	−	17
XXII. 01	Male	66	36	1	−	3
XXII. 06	Male	41	10	0	+	16
XXIX. 01 05	Male	58	19	2	−	7
XXXIII. 06 03	Male	32	18	2	+	9
XLVII 01 02	Male	[illegible]	?	0	−	11
XLVIII. 01 03	Female	41	21	0	+	13
LII. 03 06	Male	25	1	0	+	13
LIV 01 03	Male	15	1	0	+	24
LV 01 03	Female	52	36	0	+	5
LVI. 02 02	Female	26	0.5	0	−	1

of the 59 patients passed cystine-free stones in addition to pure cystine stones, three of them on two occasions.

Eighty-nine calculi were examined by chemical methods and 41 by roentgen crystallography. Chemical analysis combined with roentgen crystallography was used in the study on 13 calculi.

According to the hospital records the first calculus which 5 patients (3 male and 2 female) developed, did not contain any cystine. However it should be borne in mind that at that time only relatively rough methods were available for chemical analysis and that these calculi were analysed before the diagnosis of cystinuria was made. The possible presence of cystine may therefore have escaped attention. Unfortunately these calculi were not available for re-examination.

In a previous investigation (Hambraeus & Lagergren, 1962) 73 of the above 143 calculi derived from 34 patients with homozygous cystinuria (22 male and 12 female) were examined by roentgen crystallography and micro-roentgenography. It was found that 49 % of these calculi were pure cystine stones, in 43 % small quantities of hydroxy apatite and ammonium magnesium phosphate were present apart from cystine. In calculi cystine was absent, and in 4 calculi the predominant components were ammonium magnesium phosphate and apatite (Table 28).

Table 27 Findings on Analysis of 143 Urinary Calculi From 57 Cystinuric Patients as Given in the Hospital Records

Total urinary calculi analysed	143
By chemical methods	89
By roentgen crystallography	41
By chemical methods plus roentgen crystallography	13
Number of calculi composed predominantly of cystine	130
Number of cystine-free calculi	13

Radiographic Findings

Information about the findings on X-ray examination of the kidney was available in 75 cases (Table 29 *a*, *b* *c*). It is seen that the excretion of opaque medium was delayed unilaterally or bilaterally in 46 cases. In 21 cases there was no excretion on one side. In 17 cases the renal pelvis and ureter were shown to be dilated, and in 27 cases the renal pelvis only was dilated. In 7 cases the ureters were dilated, the renal pelvis being normal.

In 56 cases urinary calculi were revealed, being lodged in the renal pelvis in 46 cases, in the ureter in 26 cases, and in the bladder in 9 cases (Table 29 *b*) In none of these 56 cases was it stated in the hospital records that the calculi were radio-translucent. Re examination of 73 urinary calculi from 34 of the 98 patients in this series revealed that all were radio-opaque (Hambraeus & Lagergren, 1962).

In 17 cases one kidney was found to be enlarged. In 6 of them the other kidney had been removed by nephrectomy In 1 case the other kidney showed no function, in 5 cases it was smaller than normal, in 4 cases it was normal in size, and in 1 case it was congenitally absent (Table 29 *c*). In one case (not shown in the Table) one kidney showed radiographic evidence of almost certain caseo-calcareous tuberculosis.

Histo-pathological Findings

In the 9 cases in which only the macroscopic appearance of the kidney operation specimens was described, signs of pyelonephritis were seen in all and hydronephrosis of varying degrees of severity in 7

Table 28 Findings on Roentgen Crystallography and Micro-Radiology of 73 Urinary Calculi from 34 Cystinuric Patients

Findings	No. of calculi	%
Pure cystine	36	49
Cystine small amounts of hydroxyapatite and ammonium magnesium phosphate	31	43
Ammonium magnesium phosphate traces of cystine	4	6
Ammonium magnesium phosphate apatite; no cystine	1	1
Calcium oxalate; no cystine	1	1
Total	73	100

TABLE 29 *Radiographic Findings in 75 Cystinuric Patients*

	Males		Females		Total	
	No. of cases	%	No. of cases	%	No. of cases	%
(a) Excretory Urography						
Delayed excretion of medium	18	37	8	30	26	35
No excretion of medium	15	31	6	23	21	28
Poor excretion of medium	5	10	2	8	7	9
Dilated renal pelvis and ureter	10	20	7	27	17	23
Dilated renal pelvis only	17	35	10	38	27	36
Dilated ureter only	5	10	2	8	7	9
(b) *Site of Urinary Calculi*						
Renal pelvis	31	63	15	58	46	61
Ureter	17	35	9	35	26	35
Bladder	8	16	1	4	9	12
(c) *Enlargement of One Kidney*						
Compensatory following contralateral nephrectomy	5	10	1	4	6	8
Other kidney non-functioning	—	—	1	4	1	1
Other kidney small	3	6	2	8	5	7
Other kidney normal in size	2	4	2	8	4	5
Agenesis of other kidney	1	2	—	—	1	1

Microscopic re-examination of 19 kidney operation or autopsy specimens from 15 patients (Table 30) revealed the histological picture of more or less pronounced chronic pyelonephritis in 18 specimens: the peripheral portions of the renal cortex showed numerous scars which varied in size and contained shrivelled glomeruli showing hyalinisation, interstitial fibrosis, necrosis of the epithelium, and lymphocytic and plasma cell infiltration. In the central portions similar scars or focal inflammatory cell infiltration were identified which extended radially towards the apices of the papillae. The walls of the arterioles were markedly thickened their appearance resembling that of the arterioles in arteriosclerosis. In the majority of cases the large renal arteries showed arteriosclerosis of varying degree of severity (Table 30) In 2 cases (Cases Nos. XXII 06, specimen No. 2, and LI. 01 04 01) the appearance of the arteries could not be assessed because the available kidney operation specimen was too small and in 1 case (Case No. LV 01 03) assessment of their appearance was not possible owing to severe deformation of the kidney

The arteriosclerotic changes were subjectively assessed, dividing the 15 cases into the following three groups. (1) severe arteriosclerosis, (2) moderate arteriosclerosis, and (3) no evidence of arteriosclerosis. In 7 cases the arteriosclerotic changes were assessed as severe, in 2 cases as moderate,

TABLE 30 *Findings in 19 Kidney Specimens from 15 Cystinuric Patients*[a]

Case No	Sex	Age	Findings				
			Chronic pyelo-nephritis	Hydro-nephrosis	Papillary necrosis	Nephro-calcinosis	Arterio-sclerosis
IX. 02 07	Female	71	+	+			+
XXII. 01	Male	66	−	+		−	−
XXII. 06	Male	41	+	+		−	+
		56	+				
XXIII. 01 04	Female	42	−				+
		44	+				+
		46	+			−	+
XXVI. 03 11	Male	31	+	+			+
XXIX. 01 05	Male	58	+	+			+
XXXIII. 06 03	Male	32	+	+	+	+	+
XXXV 01 02	Female	27	+	+			+
XLV 01 03	Male	26	+			+	
		33	−			+	+
XLVI. 01	Male	27	+				
XLVIII. 01 03	Female	41	−	+	+		+
LI. 01 04 01	Male	48					
LV 01 03	Female	52	+			+	
LVI. 05	Female	52			+	−	+
LVI. 02 02	Female	26	−	+		+	

[a] For information of the type of these specimens see table 1

and in 4 cases as absent. In another 4 cases the available tissue specimens were too small to permit the appearance of the arteries to be assessed.

In 9 cases there was hydronephrosis. In the remaining 6 cases the possible presence of this condition could not be established because the kidney operation specimen did not include the renal pelvis (3 cases) or dilatation of the renal pelvis was not shown in serial sections (3 cases)

In 3 cases there was papillary necrosis. In 6 cases the kidney operation specimen was too small to enable one to confirm the existence of this lesion.

In 9 cases there was nephrocalcinosis associated with small, calcium phosphate containing calculi in the tubules of the kidney and focal degenerative changes of the tubular epithelium. In most cases the calculi were lodged in the distal portions of the collecting tubules, but in 4 cases calculi were also present in the proximal portions of the collecting tubules.

In 4 cases the histological findings were considered to be of sufficient interest to be described in more detail. In one of these (Case No XXVI 03 11) there was marked chronic pyelonephritis and hydronephrosis associated with severe vascular changes. Most of the glomeruli showed hyalinisation. In the areas in the kidney in which scars were absent, the glomeruli showed changes which differed from those due to arterio-

left side. He underwent left ureterolithotomy The female patient had poliomyelitis as a child with right hemiplegia. The first attack of renal colic occurred three years after the hemiplegia had subsided, the pain being experienced in the right side

In 1 case, 5 of 1. siblings had homozygous cystinuria (Family XXVI) 2 of these had a history of repeated and severe attacks of renal colic, cystitis, and pyelitis, and died at the age of 31 and 38 respectively the cause of death being given as uraemia. Two of the 5 siblings with homozygous cystinuria had a history of occasional attacks of renal colic, and 1 had never had any urinary tract symptoms. According to the information given by the siblings who were alive in 1960, the 2 deceased siblings were the only ones among them who developed severe albuminuria when the whole family contracted scarlet fever

Four male patients had diabetes. One of them was 29 years old when the disease was diagnosed. The other 3 had the first symptoms of the disease at 41 42 and 63 years of age respectively

Prognosis for Cystinuric Patients

Twenty-six patients[1] (17 male and 9 female) with homozygous cystinuria died. The causes of death and the age of the patients at death are listed in Table 32.

In 1 cases the immediate cause of death was renal failure 6 of these patients (3 male and 3 female) died between the ages of 30 and 71 years, the cause of death being given as uraemia. Of the remaining 6 patients a male patient died at the age of 28 from haemorrhage following pyelolithotomy In the other 5 cases renal failure due to urinary calculi and chronic inflammation of the kidney (glomerulonephritis or pyelonephritis) were given as the causes of death.

In cases (1 male and 1 female) cerebral haemorrhage and myocarditis with co-existent amyloidosis were given respectively as the immediate cause of death and renal failure as a contributory cause. In the female patient tuberculosis of the kidney was suggested.

In 12 cases a disease other than renal failure was given as the immediate cause of death. One male patient died of pulmonary tuberculosis at 39 years of age, 2 patients (1 male and 1 female) died of cancer with generalised metastases at the age of 75 and 77 years respectively In 6 cases cardiac disease was given as the immediate cause of death. One of these patients (male) died of coronary thrombosis, 2 patients (1 male and 1 female) of chronic myocarditis, and 3 patients (1 male and 2 female) of coronary arteriosclerosis.

In Table 33 the average life-span of the deceased cystinuric patients is given. For the 8 male patients and 4 female patients who died of renal failure, the average life-span was 37.3 years and 53 8 years respectively (The corresponding figure for the total (male + female) patients who died of renal failure, was 42.8 years.) The average life-span of the . patients in whom renal failure was a contributory cause of death was 61 years.

The average life-span of the 12 patients who died of a cause other than renal failure, was 68 6 years, that of the female patients being slightly longer (76.3 years) than that of the male patients (64 8 years)

All the 12 patients who died of renal

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[1] To the 25 cystinurics who had died by 1962, was added a further female cystinuric, who died right at the beginning of 1963

Age at Death and Causes of Death in 26 Cystinurics in This Series

	Sex	Age at death	Cause of death
	Male	34	Nephritis + nephrolithiasis
	Male	63	Cerebral haemorrhage + renal failure
	Male	39	Pulmonary tuberculosis
	Male	67	Coronary arteriosclerosis
	Female	77	Carcinoma of the breast with metastases
	Male	75	Coronary arteriosclerosis + cardiac failure
	Female	66	Chronic myocarditis + cardiac failure
	Male	75	Carcinoma of the stomach with metastases + arteriosclerosis
	Female	71	Uraemia
	Female	85	Decrepitude
	Male	86	Decrepitude
	Female	79	Coronary arteriosclerosis
	Male	68	Cardiac infarct
	Male	34	Renal failure
01	Male	43	Glomerulonephritis + nephrolithiasis
	Male	60	Chronic myocarditis
	Female	62	Uraemia
	Male	28	Haemorrhage after pyelolithotomy
	Female	30	Uraemia
	Female	59	Amyloidosis + chronic myocarditis + renal tuberculosis (?)
03 05	Male	38	Uraemia
03 11	Male	31	Uraemia
01	Male	55	Uraemia + chronic nephritis
11 01 01	Male	46	Drowning accident
02 02	Male	45	Chronic pyelonephritis + hypertension + cardiac failure
05	Female	52	Purulent pyelonephritis

...ure, were under 40 when they had their ... urinary tract symptoms. Of 1 patients ...so died of a cause other than renal failure ...ly 1 was under 40 years when urinary ...act symptoms appeared.

Medico-social Aspects of Cystinuria

Table 34 shows the distribution of the 95 cystinurics and the 57 families in which cystinuria was found to occur in the dif

TABLE 33. *Average Life-span of the 26 Deceased Cystinuric Patients*

	Males		Females		Total	
...use of death	Average life-span	No. of cases	Average life-span	No. of cases	Average life-span	No. of cases
...enal failure primarily	37.3	8	53.8	4	4..8	12
...enal failure contributory cause	63	1	59	1	61	
...use other than renal failure	64.8	8	76.3	4	68.6	12

TABLE 26. *Nephrectomy Cases*

Case No.	Sex	Age at nephrectomy (years)	Interval between appearance of symptoms and nephrectomy (years)	No. of operations for urinary calculi performed before nephrectomy	Recurrence of urinary symptoms after nephrectomy	Observation time after nephrectomy (years)
VIII. 01 03 01	Male	20	17	2	+	25
XII. 01 07	Male	44	18	3	–	4
XVI. 01 06 05	Male	35	17	2	+	24
XVII. 01 03	Female	35	23	1	–	17
XXII. 01	Male	66	36	1	–	3
XXII. 06	Male	41	10	0	+	16
XXIX. 01 05	Male	58	19	2	–	7
XXXIII. 06 03	Male	32	18	2	+	9
XLVII. 01 02	Male	21	?	0	–	11
XLVIII. 01 03	Female	41	21	0	+	13
LII. 03 06	Male	25	1	0	+	13
LIV. 01 03	Male	15	1	0	+	4
LV 01 03	Female	52	36	0	+	5
LVI. 02 02	Female	26	0.5	0	–	1

of the 59 patients passed cystine-free stones in addition to pure cystine stones, three of them on two occasions.

Eighty-nine calculi were examined by chemical methods and 41 by roentgen crystallography. Chemical analysis combined with roentgen crystallography was used in the study on 13 calculi.

According to the hospital records the first calculus which 5 patients (3 male and 2 female) developed, did not contain any cystine. However it should be borne in mind that at that time only relatively rough methods were available for chemical analysis and that these calculi were analysed before the diagnosis of cystinuria was made. The possible presence of cystine may therefore have escaped attention. Unfortunately these calculi were not available for re-examination.

In a previous investigation (Hambraeus & Lagergren, 196) 73 of the above 145 calculi derived from 34 patients with homozygous cystinuria (22 male and 12 female) were examined by roentgen crystallography and micro-roentgenography. It was found that 49% of these calculi were pure cystine stones, in 43% small quantities of hydroxy apatite and ammonium magnesium phosphate were present apart from cystine. In 2 calculi cystine was absent, and in 4 calculi the predominant components were ammonium magnesium phosphate and apatite (Table 28).

TABLE 30. *Findings in 19 Kidney Specimens from 15 Cystinuric Patients**

Case No	Sex	Age	Findings				
			Chronic pyelo-nephritis	Hydro-nephrosis	Papillary necrosis	Nephro-calcinosis	Arterio-sclerosis
IX. 02 07	Female	71	+	+			+
XXII 01	Male	66	+	+		+	+
XXII. 06	Male	41	+	+		+	+
		56	+				
XXIII. 01 04	Female	42	+				+
		44	+				+
		46	+			−	+
XXVI. 03 11	Male	31	+	+			+
XXIX. 01 05	Male	58	+	+			+
XXXIII. 06 03	Male	32	+	+	+	+	+
XXXV 01 02	Female	27	+	+			+
XLV 01 03	Male	26	+			+	
		33	+			+	+
XLVI. 01	Male	27	+				
XLVIII. 01 03	Female	41	−	+	+		+
LI. 01 04 01	Male	48	+				
LV 01 03	Female	52	+			+	
LVI. 03	Female	52			+	−	+
LVI. 02 02	Female	26	−	+		+	

* For information of the type of these specimens see table I

and in 4 cases as absent. In another 2 cases the available tissue specimens were too small to permit the appearance of the arteries to be assessed.

In 9 cases there was hydronephrosis. In the remaining 6 cases the possible presence of this condition could not be established because the kidney operation specimen did not include the renal pelvis (3 cases) or dilatation of the renal pelvis was not shown in serial sections (3 cases).

In 3 cases there was papillary necrosis. In 6 cases the kidney operation specimen was too small to enable one to confirm the existence of this lesion.

In 9 cases there was nephrocalcinosis associated with small, calcium phosphate containing calculi in the tubules of the kidney and focal degenerative changes of the tubular epithelium. In most cases the calculi were lodged in the distal portions of the collecting tubules, but in 4 cases calculi were also present in the proximal portions of the collecting tubules.

In .. cases the histological findings were considered to be of sufficient interest to be described in more detail. In one of these (Case No. XXVI. 03 11) there was marked chronic pyelonephritis and hydronephrosis associated with severe vascular changes. Most of the glomeruli showed hyalinization. In the areas in the kidney in which scars were absent, the glomeruli showed changes hich differed from those due to arterio-

TABLE 31 *Gastroduodenal Ulceration or Gastritis in 15 Cystinuric Patients*

Case No.	Sex	Age at onset of urinary symptoms	Age at onset of gastroduodenal symptoms	Type of gastroduodenal symptoms	Method of treatment
IX. 01 01 06	Male	26	33	Perforated gastric ulcer	Surgical
			45	Bleeding ulcer	Surgical
XII. 01 07	Male	26	30	Gastric ulcer (?) + chronic enteritis (ulcer not verified by radiography)	Conservative
XIV 01 02	Female	27	26	Duodenal ulceration (verified by radiography)	Surgical
XV 01 01 01	Male	12	35	Bleeding ulcer	Conservative
XVI. 01 06 05	Male	18	41	Duodenal ulceration (verified by radiography)	Surgical
XX. 01 08	Male	21	34	Bleeding gastric ulcer (verified by radiography)	Conservative
XXVI. 03 07	Male	37	?	Gastritis	Conservative
XXXII. 01 04	Male	21	28	Duodenal ulceration (verified by radiography)	Conservative
XXXVI. 01-01	Male	26	23	Gastritis	Conservative
XXXVII. 01 01	Male	29	40	Duodenal ulceration (verified by radiography)	Surgical
XLVII. 01 02	Male	20	16	Gastritis (verified by radiography)	Conservative
XLVIII 01 04	Male	22	27	Duodenal ulceration (verified by radiography)	Conservative
XLVIII. 01 09	Male	—	35	Gastritis	Conservative
LII 03 08	Female	—	31	Duodenal ulceration	Conservative
LIII. 01 03	Female	49	20	Gastritis	Conservative

sclerosis, the walls of the arterioles in the glomeruli were partly collapsed and thickened and there were extensive adhesions between the epithelium of the capillaries and the inner aspect of Bowman's capsule. Amyloid material was absent. The interstitial tissue was markedly oedematous. The major part of the epithelium of the tubules was atrophic and the lumina of the latter were filled with granular casts. These changes differed distinctly from those found in the other cases described which were regarded as being due to chronic pyelonephritis. It was strongly suggested that the changes in the kidney were due to a glomerulonephritis.

In the other case (Case No. LV 01 03) the kidney showed evidence of polycystic disease and chronic pyelonephritis.

Diseases Co-existent with Cystinuria

In 15 cases (12 males and 3 females) peptic ulcer or gastritis co-existed with cystinuria (Table 31). The clinical diagnosis of gastric or duodenal ulceration was verified by X-ray examination in 6 cases and that of gastritis in 1 case. In the remaining

9 cases the diagnosis of these conditions was based on the clinical findings and the previous history Three patients had a bleeding gastric ulcer and 1 patient had a perforated gastric ulcer

Four of these 15 patients (3 male and 1 female) were operated upon (one of them twice) for their peptic ulceration the remaining patients were treated conservatively

Eight patients developed peptic ulceration after and 4 patients before the appearance of the first urinary tract symptoms. Two patients had never had any urinary tract symptoms.

In 12 cases cystinuria was associated with cardiac or vascular disease The average age at death of 6 of the 25 deceased cystinurics was 69.5 years, the cause of death being given as cardiac disease. One of these patients (male) had a history of intermittent claudication from the age of 60

One female patient, born in 1939 had a congenital heart defect (Eisenmenger's syndrome) Of the living cystinurics who were over 40 years, 6 had shown signs of coronary disease.

Three patients (1 male and female) were treated for pulmonary tuberculosis; one of them died of this disease at 39 years of age In none of these cases was there any information vailable on the possible presence of renal tuberculosis. In 3 further cases (1 male and females) there was proven renal tuberculosis. In one of the female patients the condition had not caused any symptoms, being diagnosed on the kidney operation specimen which showed caseous changes. However the patient's brother had had pulmonary tuberculosis. The other female patient was treated in hospital at the age of 3.. because tuberculosis of the right kidney was suspected. This kidney later showed no function and at the age of 59 her urine was found to be positive for tubercle bacilli (the guinea-pig inoculation test being positive) The male patient had been treated for pulmonary tuberculosis as a child. At the age of 15 he underwent right nephrectomy because X-ray examination of the kidneys had revealed changes on the affected side (such as parenchymal calcification) which were thought to be due to tuberculosis, the presence of a coral stone being an additional finding. However it was not stated in the patient's hospital records whether or not the clinical diagnosis of tuberculosis was verified bacteriologically or microscopically

In a further case the presumptive diagnosis of renal tuberculosis was made when the patient (female) was for the first time admitted to hospital with urinary tract symptoms. She was then 41 years old. There was radiographic evidence of extensive sclerosis of the left kidney which was enlarged and irregular in outline urography revealed poor excretion of opaque medium on that side. Nephrectomy was performed and microscopy of the nephrectomy specimen revealed pyonephrosis, signs of tuberculosis or of any other specific inflammation being absent and the guinea-pig inoculation test was negative The presumptive diagnosis of renal tuberculosis was therefore discarded.

Two patients (1 male and 1 female) showed sequelae of poliomyelitis. The male patient showed paralysis of the left arm. He had his first attack of renal colic due to a urinary calculus immediately after the poliomyelitis, the pain being experienced in the

left side. He underwent left ureterolithotomy. The female patient had poliomyelitis as a child with right hemiplegia. The first attack of renal colic occurred three years after the hemiplegia had subsided, the pain being experienced in the right side.

In 1 case, 5 of 1_ siblings had homozygous cystinuria (Family XXVI) 2 of these had a history of repeated and severe attacks of renal colic, cystitis, and pyelitis, and died at the age of 31 and 38 respectively the cause of death being given as uraemia. Two of the 5 siblings with homozygous cystinuria had a history of occasional attacks of renal colic, and 1 had never had any urinary tract symptoms. According to the information given by the siblings who were alive in 1960, the 2 deceased siblings were the only ones among them who developed severe albuminuria when the whole family contracted scarlet fever

Four male patients had diabetes. One of them was _9 years old when the disease was diagnosed. The other 3 had the first symptoms of the disease at 41 4_, and 63 years of age respectively

Prognosis for Cystinuric Patients

Twenty-six patients (17 male and 9 female) with homozygous cystinuria died. The causes of death and the age of the patients at death are listed in Table 32.

In 1_ cases the immediate cause of death was renal failure 6 of these patients (3 male and 3 female) died between the ages of 30 and 71 years, the cause of death being given as uraemia. Of the remaining 6 patients a male patient died at the age of _8 from haemorrhage following pyelolithotomy In the other 5 cases renal failure due to urinary calculi and chronic inflammation of the kidney (glomerulonephritis or pyelonephritis) were given as the causes of death.

In _ cases (1 male and 1 female) cerebral haemorrhage and myocarditis with co-existent amyloidosis were given respectively as the immediate cause of death and renal failure as a contributory cause. In the female patient tuberculosis of the kidney was suggested.

In 12 cases a disease other than renal failure was given as the immediate cause of death. One male patient died of pulmonary tuberculosis at 39 years of age 2 patients (1 male and 1 female) died of cancer with generalised metastases at the age of 75 and 77 years respectively In 6 cases cardiac disease was given as the immediate cause of death. One of these patients (male) died of coronary thrombosis, 2 patients (1 male and 1 female) of chronic myocarditis, and 3 patients (1 male and 2 female) of coronary arteriosclerosis.

In Table 33 the average life-span of the deceased cystinuric patients is given. For the 8 male patients and 4 female patients who died of renal failure, the average life-span was 37.3 years and 53.8 years respectively (The corresponding figure for the total (male + female) patients who died of renal failure, was 4_8 years.) The average life-span of the 2 patients in whom renal failure was a contributory cause of death, was 61 years.

The average life-span of the 12 patients who died of a cause other than renal failure, was 68 6 years, that of the female patients being slightly longer (76.3 years) than that of the male patients (64.8 years)

All the 12 patients who died of renal

To the 25 cystinurics who had died by 1962, was added further female cystinuric, who died right at the beginning of 1963.

TABLE 32. *Age at Death and Causes of Death in 26 Cystinurics in This Series*

Case No.	Sex	Age at death	Cause of death
I. 01	Male	34	Nephritis + nephrolithiasis
II. 01	Male	63	Cerebral haemorrhage + renal failure
III. 01 01	Male	39	Pulmonary tuberculosis
IV 05	Male	67	Coronary arteriosclerosis
VI. 01 02	Female	77	Carcinoma of the breast with metastases
VI. 01 04	Male	75	Coronary arteriosclerosis + cardiac failure
VI. 01 07	Female	66	Chronic myocarditis + cardiac failure
VII. 01	Male	75	Carcinoma of the stomach with metastases – arteriosclerosis
IX. 02 07	Female	71	Uraemia
X. 06	Female	85	Decrepitudo
XI. 01	Male	86	Decrepitude
XII. 01 02	Female	79	Coronary arteriosclerosis
XII. 01 07	Male	68	Cardiac infarct
XIV 01 01	Male	24	Renal failure
XV 01-01 01	Male	43	Glomerulonephritis + nephrolithiasis
XVIII. 01	Male	60	Chronic myocarditis
XIX. 02	Female	62	Uraemia
XIX. 03	Male	28	Haemorrhage after pyelolithotomy
XIX. 05	Female	30	Uraemia
XXI. 05	Female	59	Amyloidosis + chronic myocarditis + renal tuberculosis (?)
XXVI. 03 05	Male	38	Uraemia
XXVI. 03 11	Male	31	Uraemia
XXXI 03	Male	33	Uraemia + chronic nephritis
XXXVII 01 01	Male	46	Drowning accident
XLIX. 02 02	Male	45	Chronic pyelonephritis hypertension + cardiac failure
LVI 05	Female	52	Purulent pyelonephritis

failure, were under 40 when they had their first urinary tract symptoms. Of 12 patients who died of a cause other than renal failure only 1 was under 40 years when urinary tract symptoms appeared.

Medico-social Aspects of Cystinuria

Table 34 shows the distribution of the 95 cystinurics and the 57 families in which cystinuria was found to occur in the dif

TABLE 33 *Average Life-span of the 26 Deceased Cystinuric Patients*

Cause of death	Males		Females		Total	
	Average life-span	No. of cases	Average life-span	No. of cases	Average life-span	No. of cases
Renal failure primarily	37.3	8	53.8	4	42.8	12
Renal failure contributory cause	63	1	59	1	61	2
Cause other than renal failure	64.8	8	76.3	4	68.6	1

TABLE 34 *Distribution of Cystinurics and "Cystinuric Families" in the Different Counties of Sweden in 1960*

County Reference letter	Name	No of inhabitants	No. of cystinurics Alive	Dead	Total	No. of cystinuric families	No. of living cystinurics per 100,000 inhabitants
A	Town of Stockholm	806,903	12	3	15	14	1.5
	County of						
B	Stockholm	462,938	2	—	2	2	0.4
C	Uppsala	167,856	1	—	1	1	0 6
D	Sodermanland	227 924	1	1	2	2	0 4
E	Östergötland	357 785	2	2	4	3	0 6
F	Jönköping	285 401	1	—	1	1	0.4
G	Kronoberg	159,094	1	—	1	1	0 6
H	Kalmar	235 635	1	—	1	1	0 4
I	Gotland	54,209	—	1	1	1	—
K	Blekinge	144 498	3	1	4	2	2.1
L	Kristianstad	256,541	6	5	11	8	2.3
M	Malmöhus	629 116	8	—	8	4	1.3
N	Halland	170 011	1	1	2	2	0 6
O	Göteborg and Bohus	625,366	3	1	4	4	0.5
P	Älvsborg	373,037	3	2	5	5	0 8
R	Skaraborg	249,901	5	2	7	3	2.0
S	Värmland	291,026	7	—	7	3	2.4
T	Örebro	262,534	2	—	2	2	0.8
U	Västmanland	232,966	2	2	4	3	0.9
W	Kopparberg	486,309	—	—	—	—	—
X	Gävleborg	293,385	2	—	2	2	0.7
Y	Västernorrland	283,745	4	—	4	2	1 4
Z	Jämtland	139,918	2	—	2	2	1 4
AC	Västerbotten	239 704	2	3	5	2	0 8
BD	Norrbotten	261 958	1	—	1	1	0 4
Total Sweden		7 498,770	72	24	96		0.96

ferent Counties of Sweden as traced in 1960. It is seen that the number of cystinurics who were alive when this work was in progress varied from County to County whilst the number of cystinuric families varied to a lesser degree. The largest number of cystinurics was found in the Counties of Blekinge, Kristianstad, Skaraborg, and Värmland, whilst in the Counties of Gotland and Kopparberg no living person with cystinuria had been traced up to 1960.

The incidence of living cystinurics in Sweden was 0.96 per 100,000 inhabitants.

Table 35 shows the civil status of the patients and the number of patients who had children and were childless respectively

Of 29 married female patients 25 had between 1 and 9 children (average number 2). One married female patient died child

TABLE 35 *Civil Status and Number of Children of 93 Cystinurics*

Civil Status	Males				Females			
	Dead		Alive		Dead		Alive	
	No. of cases	Average age (years)	No. of cases	Average age (years)	No. of cases	Average age (years)	No. of cases	Average age (years)
Single and childless	5	32.8	12	28.7	2	66.5	9	24.7
Single with children	—	—	—	—	—	—	1	29
Married but childless	1	60	4	48 3	1	75	3	48.3
Married with children	10	61.2	22	45.2	5	63.8	20	46 4
Total	16	51.8	38	42.2	9	65.3	32	44.4

Three cases were excluded because information on their civil status was not available.

less at the age of 75 3 married female patients who were alive in 196.., were 30 54 and 55 years old respectively and had no children. One living single female patient aged 9 years, had one child. Of the remaining 9 single female patients 2 were over 50 years of age and 7 were between the ages of 9 and 21 (average age 15.3) in 1962

Of 57 male patients 37 were married, and 17 were single, and in 3 cases no information about the civil status was available Of the 37 married male patients 32 had between 1 and 10 children, the average number of children per male patient being 2 5 patients had no children. According to the hospital records of these latter patients were voluntarily childless. F ve of the unmarried male patients were dead in 196.., their age at death being stated to range between 24 and 43 years (average age 3..8 years)

Information about the patients fitness for military service was available in 20 cases 16 had done their military service and 4 had been exempt from it on account of their cystinuria. In 1 case in the former group the first attack of renal colic had occurred and the diagnosis of cystinuria had been made before the patient had enlisted 5 patients had their first urinary tract symptoms during their first period of

TABLE 36 *Distribution of the 90 Cystinuric Patient Above 20 Years in the Different Occupational G oups*

Occupations	
Males	
Agricultural, forestery fishery and road labourers	9
Industrial workers and craftsmen	15
Workers in Trade and Commerce	8
Public service employees and selfemployed men	16
Student	1
Occupation unknown	5
Total	54
Females	
Married	27
Gainfully and selfemployed	7
No occupation	2
Total	36

TABLE 37 *Number of Days 39 of 68 Cystinurics over 16 Years Were on the Sick-list or in Hospital Care from 1956 to 1960*

County References letter	Name	No. of cystinurics	Average no. of days of sickness per year per cystinuric[a]	Average no. of days in hospital per year per cystinuric[a]
A	Town of Stockholm	5	[illegible]3.4	8.2
	County of			
B	Stockholm	1	12	10.6
C	Uppsala	1	1.8	—
D	Södermanland	—	—	—
E	Östergötland	1	12.6	6.6
F	Jönköping	1	22	3.6
G	Kronoberg	1	15	6.8
H	Kalmar	—	—	—
I	Gotland	—	—	—
K	Blekinge		22	6.5
L	Kristianstad	3	35.4	12
M	Malmöhus	2	36.8	12.5
N	Halland		22.4	13.8
O	Göteborg and Bohus	1	17.2	3
P	Älvsborg	2	[illegible]8.7	18
R	Skaraborg	3	53.8	11.8
S	Värmland	2	65.5	[b]
T	Örebro	1	9.4	3.2
U	Västmanland		162.5	[b]
W	Kopparberg	—	—	—
X	Gävleborg	1	20.8	12.6
Y	Västernorrland	2	32.5	[b]
Z	Jämtland	1	18.2	5.8
AC	Västerbotten	2	53.9	7.8
BD	Norrbotten	1	2.8	—
In the whole of Sweden		39	34.2	9.6

[a] On account of cystinuria or complications. [b] No information available.

military service and 10 patients did not have any symptoms during this period.

The 4 patients who were exempt from military service, had had repeated attacks of renal colic before being called up. One of these patients passed a medical examination later on.

Five patients were alcoholics. The Temperance Council had taken action against 3 of them. One of the latter patients was a drug addict in addition and had been treated by a psychiatrist, another had several times been treated as an in-patient, and the third as an out-patient in an institution for the care of alcoholics. All patients in this group had had repeated attacks of renal colic and had been operated upon at least once.

The occupational status of the patients

varied greatly (Table 36) A relationship between the type of occupation and the degree of severity of cystinuria could not be demonstrated.

The capacity for work was unaffected in all cases but 3 One of these latter patients received an invalidity pension from the age of 43 Another had received sickness benefit for 717 days, from the age of 48 until his death at 55 and the third, a factory hand, for 475 days from the 1st of January 1956 until the 1st of June 1962. This third patient later trained to be a cook because the work at the factory had become too hard for him.

Table 37 shows the average number of days for which 39 of 68 patients (over 16 years) had received sickness benefit or were treated in hospital on account of cystinuria or its complications in the years from 1956 to 1960, the figures being quoted from the records of the Central Sickness Benefit Society

It is seen that the average number of days for which sickness benefit was paid per cystinuric patient per year was 34.2 days, the average number of days of hospital treatment being 9 6 per patient per year during this period.

In three Counties the information obtained about the duration of the patients hospitalisation was uncertain. These patients were therefore excluded.

According to the records of the Central Sickness Benefit Society the total number of days for which sickness benefit was paid to persons affected with cystinuria or its sequelae was 6,434 in the years from 1956 to 1960 the total number of days for which these patients were in hospital during this period being 1,367 (the patients resident in the three Counties in which the information obtained about their hospitalisation was unreliable being excluded)

DISCUSSION

Evaluation of Clinical Data

The information afforded by the hospital records, varied greatly: in some only a brief account of the patient's condition was given, while in others the clinical and laboratory findings were fully reported. For this reason the figures referring to the incidence of the clinical and laboratory findings in the cystinurics probably represent the minimum incidence (Table 13 and 15).

Causes of Death

Berfenstam & Linne (196.) and Otterland (1962) compared the parish register records of the causes of death of Swedish subjects in general with those stated in the death certificates and found them valid and accurate. As the data on the causes of death in the cases in this series were quoted from the parish registers it may reasonably be assumed that they were authentic.

In the cases in which the data on the patients' families were incomplete, supplementary information was obtained from the parish registers concerned.

Sickness Benefit Grants

From 1955, when the National Health Insurance Act came into force, the periods for which every Swedish subject (over 16 years) has been in private medical care or in hospital, i. e. has received sickness benefit, have been recorded in the registers of the local Sickness Benefit Societies in the different Counties of Sweden. As the corresponding data on the cystinuric patients in this series given for the years 1956 to 1960 were quoted from these registers it may be assumed that they are correct.

The Diagnostic Value of the Analytical Methods Used

The presence of cystine crystals in the urinary sediment indicates an increased output of cystine in the urine and this is suggestive of cystinuria. However, it has been reported (Ultzmann, 1871, Link, 1912, Lewis, 1932) that cystinuria may occur *without* the appearance of cystine crystals in the urinary sediment. Microscopy of the urinary sediment alone should not therefore be regarded as the only diagnostic test in screening examinations performed for the detection of cystinuria.

Re-examination of urinary specimens from 17 cystinurics and 92 relatives of these patients in Mörner's series confirmed Mörner's findings. It may therefore be assumed that none of the remaining 60 relatives of these patients, specimens of whose urine were not available for re-examination by us and whose excretion of cystine Mörner had found to be normal, had homozygous cystinuria.

Brand's test (Brand *et al.* 1930) gives a positive reaction in cases in which the urinary excretion of cystine is abnormally high. However, this test is not specific for cystine. It also gives a positive reaction with

compounds which include a sulphydryl group (SH) or a group which cyanide reduces to a sulphydryl group. This test has been widely used in screening examinations (Lewis, 193_, Dent & Harris, 1951 Boström & Tottie 1959) It is of value in differentiating between normal individuals and cystinurics (Brand *et al.*, 1930· Dent & Harris, 1951 Harris & Warren, 1953 Harris & Robson, 1957), but it does not enable the differential diagnosis to be made either between semi-cystinuria and homozygous cystinuria (Harris & Warren, 1953 Boström & Tottie 1959) or between persons with a *mild* form of semi-cystinuria and normal persons (Harris & Warren, 1953)

In homozygous cystinuria the urinary excretion of the basic amino acids lysine, arginine, and ornithine is increased in addition to that of cystine (Dent & Rose, 1951 Dent & Harris, 1951 Stein, 1951 Harris *et al.*, 1955 *a*). Two-dimensional paper chromatography permits the demonstration of an increased output in the urine of cystine and the basic amino acids, but it is of little value in the differential diagnosis between homozygous cystinuria and semi-cystinuria, because it does not result in satisfactory separation of the individual basic amino acids. Paper electrophoresis permits the separation of the latter and therefore enables differential diagnosis between these two conditions to be made with assurance (Harris & Warren, 1954 Harris *et al.*, 1955 *a*· Hambraeus, 1961).

Mörner (1922 *b* 1926 1932) reported that quantitative studies of the concentration of cystine in the urine from 20 cystinurics revealed values between 0.025 g and 1 15 g per litre of urine the average value being 0.51 g per litre of urine. In only 2 cases were values below 0 22 g per litre of urine recorded. These values agree with those obtained by modern analytical methods such as microbiological assay (Dent & Rose, 1951 Dent *et al.*, 1954 *a* Harris *et al.*, 1955 *a*) polarography (Reed, 1942, Fowler *et al.*, 1952 Harris & Warren, 1953 Dent *et al* 1954 *b* Arrow & Westall, 1958) and ion exchange chromatography (Stein, 1951 Evered, 1956 Cusworth & Dent, 1960· Hambraeus, 1964 *a*).

If urinary calculi are found to contain cystine, cystinuria is suggested. With the exception of a few cases of Fanconi's syndrome and semi-cystinuria (Harris *et al* 1955 *b* Harris & Robson, 1957 Hambraeus, 1964 *b*) urinary calculi composed *predominantly* of cystine have been found in cases of homozygous cystinuria only

The chemical analyses of the available urinary calculi were carried out by a large number of investigators, and the qualitative and quantitative methods used varied greatly whilst the *same* technique was invariably used in roentgen crystallography of the urinary calculi (Lagergren, 1956· Hambraeus & Lagergren, 196_) The authors are therefore inclined to believe that this method provided information of greater diagnostic value in this investigation than did the chemical analyses, with the exception of Mörner's quantitative studies.

Accuracy of the Diagnosis

In the 77 cases in which urinalysis was performed, using Brand's test combined with paper chromatography and paper electrophoresis, the diagnosis may be considered as correct.

In 3 of the remaining 1 cases the patients had died before this investigation was

begun and the diagnosis of cystinuria was based on the past histories of these patients (which was characteristic of the disease) and on the occurrence of homozygous cystinuria among their siblings. In 2 of these cases the macroscopic appearance of the urinary calculi, as described in the hospital records suggested that they were cystine stones.

In a further 13 cases in which the patient was dead at the time of this investigation, Mörner had found the concentration of cystine in the urine to be abnormally high. Ten of these patients had formed cystine stones. In the 3 other cases in this group stone analysis had not been carried out or the patient had not formed any urinary calculi. One of these patients had not had any urinary tract symptoms. Mörner found that this last patient excreted 0.49 g of cystine per litre of urine. The diagnosis may therefore be regarded as correct in this case also.

In 5 cases the diagnosis was based mainly on the demonstration of cystine stones. In 2 of these patients cystine crystals had been identified in the urinary sediment in addition, and in another 2 homozygous cystinuria had been found to occur among their siblings.

In 2 cases in Mörner's series in which the patient had died, Mörner had found very small amounts of cystine in the urine, viz. 0.05 g and 0.025 g per litre of urine respectively. One of these patients had formed a vesical stone which was found to be composed of cystine and the other had passed stones spontaneously which were not analyzed. However in the latter patient the analysis of a specimen of urine, passed following an attack of renal colic, revealed cystine crystals in the urinary sediment, and the present investigation disclosed the occurrence of semi-cystinuria in the patient's family. As Mörner had regarded these 2 patients as cystinurics and in view of the fact that the present investigation follows up Mörner's studies, it was thought permissible to include them, although the argument may be raised that they had semi-cystinuria rather than homozygous cystinuria.

Incidence of Cystinuria in Sweden

According to the literature the incidence of cystinuria in a normal population is 1 : 10 000–20 000 (Simon, 1900; Primavera quoted by Garrod 1909; Sondern, 1911). These figures were based on the demonstration of cystine crystals in the urinary sediment. As already mentioned cystinuria may be present *without* the appearance of cystine crystals in the urinary sediment. For this reason, the above incidence of the disease is probably too low.

Lewis (1932) investigated the urine from about 10,000 healthy American students, using Brand's test (Brand *et al.*, 1930) and Sullivan's test (Sullivan, 1929) and found the urinary excretion of cystine to be abnormally high in 1 : 600. This incidence probably does not hold good for homozygous cystinuria as Brand's test also gives a positive reaction in cases in which the urinary output of cystine is only slightly increased. As already mentioned, in a screening examination of 7 793 school children in Stockholm who were all of the same age, Boström & Tottie (1959) found 3 to have homozygous cystinuria, *i.e.* 1 in 2,598. On the basis of these figures thousands of Swedish subjects should have cystinuria. However this investigation has shown that

not more than 98 cases of cystinuria were diagnosed in the years from 1870 to 196_. There are probably many factors concerned in the discrepancy between the expected incidence and the one actually determined during that period.

As the diagnosis of cystinuria has almost exclusively been based on the findings on stone analysis, cystinuria may have been overlooked for the following reasons:

(1) Urinary calculi may not invariably have been available for analysis.

(2) Stone analysis may not always has been carried out in the different hospitals.

(3) Cystinurics may occasionally pass mixed stones or stones composed f elements other than cystine (Hambraeus & Lagergren, 196_). If qualitative analysis alone is carried out in such cases traces of cystine may easily escape attention.

In the majority of cases in this series the cystinuria was diagnosed in the years from 1921 to 1930 or from 1951 to 1962. During these two decades unusual attention was paid to cystinuria, probably stimulated by Mörner's studies and the present work.

Sex Distribution

As regards the sex distribution the number of males was slightly larger than that of females, *i.e.* 57 as against 41. The predominance of male patients over female patients in cases of cystinuria has been observed by numerous authors (Niemann, 1876, Simon, 1900, v. Hofmann, 1907, Link, 1912, Kretschmer, 1916, Garrod, 1923, Lewis, 1932, Mörner 19__ b 1926, 193_, and others). In the present series it appears to be due to the larger number of male patients in the primary cases, *i.e.* 38 males as compared with 17 females. In the secondary cases there was no noteworthy difference between the number of male and female patients, *i.e.* 17 as compared with _1.

On the basis of the above observations the argument may be raised that the higher incidence of cystinuria in males reported in the literature, is not genuine and genetically determined. The present authors support the view expressed by Niemann (1876), Simon (1900), and Link (191_) that the symptoms arising from the formation and passage of calculi owing to predisposing anatomical factors are severer in character in male cystinurics than in female cystinurics. The former generally seek medical advice earlier and oftener and this results in cystinuria being more frequently recognised in males than in females. However if the diagnosis of cystinuria is made no matter whether urinary tract symptoms are present or not (as in the secondary cases), there is no difference between the sexes in respect to the incidence of cystinuria. Harris & Warren (1953) and Harris *et al.* (1955 *b*) have made the same observation.

Clinical Aspects of Cystinuria

The clinical picture of cystinuria has been much discussed in the medical literature since 1810 when Wollaston described the first case of cystinuria, the discussions being based either on the cases already published or on personal observations.

Niemann (1876) was the first to review the clinical features in 5_ cases of cystinuria published in the literature, adding the observations made on one personal case. Although he had personally observed only one case and had no data available other than those published in the literature, he gave an illustrati survey of the cardinal

clinical features of the disease. His paper was followed by numerous reviews of cases of cystinuria described in the literature (Simon, 1900; Neuberg, 1907; Link, 1912; Kretschmer, 1916; Mörner, 1920; Renander, 1941; and others).

As already mentioned the first extensive studies on cystinuria based on personal experience, were carried out by the Swedish investigator Mörner (1924–1937). Since then Dent, Harris, and their collaborators have published a series of papers on cystinuria (Dent & Rose, 1951; Dent & Harris, 1951; Harris & Warren, 1953; Dent *et al.*, 1954 *a*, *b*; Dent & Senior, 1955; Harris *et al.*, 1955 *a*, *b*; Robson & Rose, 1957). They reported on 37 cystinuric patients who were individuals from 21 families, 232 members of which were examined. These workers made notable contributions to the elucidation of the aetiology and the genetics of the disease and its treatment.

The 98 cystinuric patients in this series, who were individuals from 59 families, constitute, to the best of the author's knowledge, the largest personal series of cases which has hitherto been published. As the follow-up of these patients was longer than in any other case material published, conditions were particularly favourable for studying the clinical course and the complications of cystinuria and any co-existent disease.

It has been found that the only symptoms associated with classical cystinuria are those arising from the development of urinary calculi.

The observation has been confirmed that renal colic or spontaneous passage of calculi are the presenting symptoms in the majority of cases. However, it is interesting to note that of the 81 cases in which the presenting symptoms were recorded, the latter were due to pyelitis, pyelocystitis, and cystitis in as many as 21. Little attention has so far been given to the possibility that urinary tract infection may give rise to the presenting symptoms. Dent & Senior (1955) claimed that urinary tract infection is of minor importance in lithiasis in cystinurics. However, on the basis of the observations made in this work the present authors are inclined to believe that, as with the formation of urinary calculi in general (Boshamer *et al.*, 1961), urinary tract infection is a causative factor in the development of cystine stones.

The view expressed by Niemann (1876), Stadthagen & Brieger (1889), Simon (1900), Link (1912) and others that cystinurics may form urinary calculi at any age, has been confirmed. Kretschmer (1916) reviewed 107 cases from the literature and found that 73 % of these patients had experienced the first urinary tract symptoms by the age of 40. In the present series the corresponding percentage was slightly higher (80 %).

The incidence of lithiasis in the younger age groups in this series is in agreement with that reported by Niemann (1876), i.e. 7 % of the 88 cystinuric patients were under 10 years when they had their first attack of renal colic, the corresponding percentage in the cases reviewed by Niemann being 10 %. In the latter series as well as in the present investigation all these patients were males.

It was found that the male patients had the first symptoms of the disease at an earlier age than the female patients. This is in agreement with the observation that the disease causes greater discomfort to male patients, being therefore recognised

in males earlier than in females. It has also been observed that the first symptoms appear earlier in the primary cases than in the secondary cases.

As there was practically no difference between the primary and secondary cases with respect to the incidence of lithiasis and the clinical course of cystinuria, it was considered unnecessary to discuss them separately. The two groups were therefore combined, the findings being shown in Fig. 1. It is seen that 91% of the 98 patients had urinary tract symptoms on one or more occasions. This observation supports the view expressed by Niemann (1876) that the majority of cystinurics sooner or later develop urinary calculi.

Dent & Senior (1955) reported that of 15 patients whom they had observed and in whom the diagnosis was made on chemical urinalysis alone, 8 eventually formed stones. This observation led them to assume that at least 50% of cystinurics developed urinary calculi. As these 15 cases (which they termed "unbiased cases") were cystinurics detected at screening examinations of families in which cystinuria was known to occur, they are fully comparable with the secondary cases in the present series. The incidence of urinary tract symptoms in the 33 secondary cases was 76%; the corresponding percentage in the secondary cases followed up for more than 40 years being 95% (Tables 18 and 20). It is open to discussion whether the incidence of urinary tract symptoms is equally high in cases of cystinuria discovered by random screening examinations of a "normal" population.

About 50% of the patients in this series developed urinary tract infection sooner or later in the course of cystinuria. Link (1912), and others claimed that urinary tract infection was quite common in cases of cystinuria. According to Harris & Robson (1957), however, the incidence of urinary tract infection in these cases is remarkably low.

With the exception of a few cases in which one of us (H.) personally measured the blood pressure, the readings were quoted from the hospital records. As the blood pressure of these patients was measured by many different people who probably used different techniques and instruments of varying quality, the readings did not permit one to draw any definite conclusions.

The patients appeared to fall into the following three categories:

(1) Patients with a blood pressure less than 160/100 mmHg. The majority had always had a low blood pressure and had never had either subjective or objective manifestations of hypertension.
(2) Patients whose blood pressure varied between 160 mmHg and 180 mmHg systolic and 100 mmHg and 110 mmHg diastolic on one or more occasions. Some of these patients had a labile blood pressure and/or mild objective features of hypertension.
(3) Patients whose blood pressure exceeded 180/100 mmHg on one or more occasions and who had subjective and objective symptoms of hypertension.

Hypertension was a comparatively late feature irrespective of whether it was due to cystinuria *per se* or of unknown cause. There was no difference between the sexes regarding the incidence of hypertension.

The view was confirmed that cystinurics generally formed calculi bilaterally. This is not surprising as the cause of cystinuria

is a congenital defect of renal tubular re absorption. On the other hand Brausch (1933) claimed that a characteristic feature of the disease was that calculi formed on one side only However this view may be regarded as disproved.

Bilateral symptoms were more common in the male patients and the primary cases than in the female patients and secondary cases. This is further evidence in support of the view that cystinuria is more severe in character in males than in females.

It has been reported that the site of the urinary calculi varies. Bell (1946) *e g* found that they occurred on the left side in the majority of his cases. In the present investigation the site of the calculi varied in both sexes, particularly in the female, but the difference was not statistically signifi cant.

The observation that recurrent stone formation and the spontaneous passage of calculi are characteristic features of cystinuria (Hammer & Thompson, 1940 Dent & Rose, 1951 and others) was confirmed. 58 % of the patients passed urinary calculi spontaneously many of them passing more than 100 small calculi. In 89 % there was a history of repeated stone formation, the incidence of recurrence being slightly higher in the male patients.

Surgical Procedures Used for the Removal of Urinary Calculi

Many of the patients in this series were still young when they were operated upon for urinary calculi Fewer females than males underwent surgery The probable explanation for this observation lies in the anatomy of the female lower urinary tract which permits calculi to pass spontaneously with greater ease than does the lower urinary tract in the male. Surgical procedures for the removal of vesical calculi were rarely performed in women. This was probably a factor in the difference between the males and females in this series with regard to the average number of opera tions performed per patient, the average being greater in the male patients.

There are several explanations for the comparatively large number of operations performed on the individual patients: (1) the marked tendency of cystinurics to form urinary calculi (2) failure to remove *all* calculi at operation, (3) post-operative scar formation interfering with the secretion and voiding of urine, further stone formation being thereby encouraged, (4) a post-operative disturbance of the patient's fluid balance resulting in reduced secretion of urine· (5) post-operative tissue necrosis perhaps contributing to increased urinary excretion of cystine and (6) post-operative or chronic urinary infection, which may give rise to renal lithiasis (Hellström, 1936 Carroll & Brennan, 195)

The higher incidence of operations in the primary than in the secondary cases may have been due to the fact that the former patients all had urinary tract symptoms which made them seek medical advice, whilst this did not invariably apply to the latter patients.

The diagnosis of cystinuria was usually made after the initial operation, but in quite a number of cases the condition was not recognised until after several operations had been performed. The possible presence of cystinuria should, therefore, be borne in mind and the urine should always be tested for cystine in patients with recurrent urinary tract symptoms.

Of the 8 cases in which the diagnosis

was made before the patient was operated on, there was a history of recurrent urinary tract symptoms and repeated spontaneous passage of calculi in 6 cystinuria being revealed by stone analysis. The remaining 2 patients belonged to the group secondary cases.

Some Aspects of Nephrectomy

Nephrectomy is of special clinical interest because it differs from the other operations on the urinary tract inasmuch as it is performed to remove not only urinary calculi but also a damaged kidney the continued presence of which might have given rise to either recurrent stone formation or hypertension or both.

The age of the patient and the duration of his symptoms prior to making the diagnosis varied in the nephrectomy cases. There probably is a relationship between the length of the post-operative observation time and the number of cases of recurrence of urinary tract symptoms observed.

As recurrent stone formation may give rise to hydronephrosis and chronic pyelonephritis, which damage the renal parenchyma, every effort should be made to preserve as long as possible as much functioning parenchymal tissue as possible

As nephrectomy does not appear to have any therapeutic effect on cystinuria, the operation is not indicated in case of unilateral urinary tract symptoms.

In 3 nephrectomy cases microscopy of the operation specimens revealed the histological picture of relatively mild pyelonephritis. As there was no history of impaired renal function or of a previous operation to remove urinary calculi, the argument may be raised that nephrectomy was not indicated in these cases.

Stone Analysis

Most of the cystinurics in this series formed more or less pure cystine stones but a few formed cystine-free stones in addition. In reviewing the literature on cystinuria Mörner (19 0) found that already Wollaston (1810) had encountered a case of cystinuria in which the patient had formed a cystine stone on one occasion and a stone composed of uric acid on another occasion. Since then several cases of cystinuria have been reported in which the patient formed cystine-free stones (Mörner 1922 b 1932 Stallman, 1925 Ewell, 1932, Pollak, 1934 Herman & Lee, 1935). This observation was confirmed in an earlier investigation (Hambraeus & Lagergren, 1962) and in the present work. In 5 cases in the present series the hospital records of the analysis of the first calculus the patient had passed, made no mention of cystine. As cystinuria had not yet been diagnosed in these cases at that time the possibility cannot be excluded that the presence of small amounts of cystine in these first calculi might have been overlooked.

Radiographic Findings

Urography revealed that urinary calculi caused impaired renal function in the majority of cases on one or more occasions, *i.e.* in some cases there was no excretion of opaque medium, while in others excretion was delayed or there was radiographic evidence that the ureters and/or the renal pelvis were dilated.

Obstruction of a part of the urinary tract may affect the kidneys in two ways. (1) it increases the risk of infection (Boshamer *et al.* 1961) and ( ) blockage of the ureters increases the pressure within the

kidney and this may result in a disturbance of the function of the renal cells (Widén, 1958) A disturbance of the function of the renal cells may have been the cause of the non-functioning kidney in Case No. XXXVIII 02 04

The view expressed by numerous workers (Morris, 1906 Winsbury White, 1924 Henline, 1930 Morrison, 1940 Renander 1941 Gouverneur 1950 Parsons, 1952) that cystine stones are radio-opaque was confirmed in a previous investigation (Hambraeus & Lagergren, 1962) and again in this work. Failure to visualize them in earlier investigations (Seeger & Kearns, 1925 Hicks, 1931 Ewell, 1932 Hume 1932 Herman & Lee 1935) might have been due to technical factors.

Histo-pathological Aspects

The renal tissue specimens available for histological examination did not permit any definite conclusions to be drawn as to the possible relationship between the duration and severity of the clinical symptoms and the extent of the morphological changes for the following reasons. Firstly the specimens obtained at biopsy or partial nephrectomy were from severely damaged parts of the kidney in some cases and from seemingly intact parts in others, autopsy specimens of both kidneys being available in 3 cases only Secondly the patient's age at the time when the renal tissue specimens were obtained varied greatly Lastly the intervals between the appearance of the initial symptoms or urinary tract infection, the establishment of the diagnosis of cystinuria, and operative procedures also varied from one case to the other

The vascular changes in the kidney were virtually identical with those characterizing arteriosclerosis, but were remarkably severe in many cases. The question arises whether these changes occur independently of inflammatory changes or whether the generally accepted view that they are due to inflammation is correct. The degree of severity of the arteriosclerotic changes was proportional to that of the cystinuria in patients who were younger than 35 years when the renal tissue specimens were obtained. This suggested that the vascular changes were primarily due to cystinuria and its complications.

In the 2 cases in which microscopy of the kidney specimens revealed severe nephrosclerosis, the patients had formed cystine free stones and mixed stones. It is not possible to draw any definite conclusions as to the cause of the nephrosclerosis on the basis of the occurrence of these stones. A relationship between the degree of severity of the inflammatory changes and the nephrosclerosis could not be demonstrated.

Diseases Co-existent with Cystinuria

Medical literature affords little information on diseases co-existing with cystinuria. The investigation of these diseases presented difficulties in the cases in this series because a good many of the available hospital records did not afford any information on this point. However it would seem that cystinurics tend to develop gastro-duodenal ulceration as 21 % of the male and 7 % of the female patients in this series had such a history According to the literature the incidence of gastro-duodenal ulceration varies greatly (Hellström 1958/59). Tomenius (1955) investigated the incidence of peptic ulceration among the patients of the

hospitals in Stockholm in the years from 1938 to 1952 and found it to be 45–66 in 10,000 for male patients and 15–[illegible]0 in 10 000 for female patients. The figures reported by Pulvertaft (1958) were in agreement with these figures. According to Jennison (1938) and Doll (1952), however the incidence is much higher *i.e.* 2–10%. In a study of 1[illegible]1 cases of hyperparathyroidism Hellström (1958/59) found the incidence of peptic ulceration to be [illegible]8 6 per cent in male patients and 4 6 per cent in female patients. On the basis of these figures (which are in agreement with the corresponding figures in this series) Hellström assumed that patients with hyperparathyroidism are very prone to develop gastro-duodenal ulceration. The analogy between Hellström's observations and those made in the present series is interesting as patients with hyperparathyroidism and patients with cystinuria both tend to form urinary calculi.

There was one case of congenital heart disease (Eisenmenger's syndrome). This case and another one in which one kidney was congenitally absent, illustrate that cystinurics may occasionally show congenital anomalies. The same observation was made by Dent & Harris (1951) who reported a case of cystinuria associated with haemophilia, and by Hambraeus & de Hevesy (1964), who described a case in which coeliac disease co-existed. However no definite conclusions can be drawn from these sporadic cases.

In 4 cases renal tuberculosis was suspected, being bacteriologically confirmed by the guinea-pig inoculation test in 1. In the remaining 3 cases the diagnosis of tuberculosis was in the main based on radiographic evidence of extensive nephrosclerosis.

It has been reported that patients who suffer from the sequelae of poliomyelitis, tend to form urinary calculi usually calcium oxalate stones (Pierre, 1953 and others). The tendency towards stone formation of such patients has been regarded as being due to hypercalcuria secondary to an osteoporosis of disuse associated with the patient's immobilisation (Albright & Reifenstein, 1948). In one of the [illegible] cases of poliomyelitis in this series the patient had also formed a stone predominantly composed of calcium oxalate.

Life-span and Causes of Death

In about half of the cases in which the patient died, the immediate cause of death was renal failure, the renal failure being due to complications of cystinuria such as pyelonephritis or urinary tract obstruction. There was no difference between the sexes as far as the immediate cause of death was concerned.

It was found that the average life-span of the cystinurics who died of renal failure, was 20–30 years shorter than that of the patients who died of a cause other than renal failure. This applied to both sexes in both groups, but the average life-span of the male patients was about 10 years shorter than that of the female patients in both groups.

The complications of cystinuria seriously affected the prognosis for the patients. The life-span of no less than 50% of the patients who died of complications of cystinuria was about 25 years shorter than normal. Effective prophylaxis against infection and hydronephrosis and the preservation of as much functioning renal parenchyma as possible is therefore of major importance.

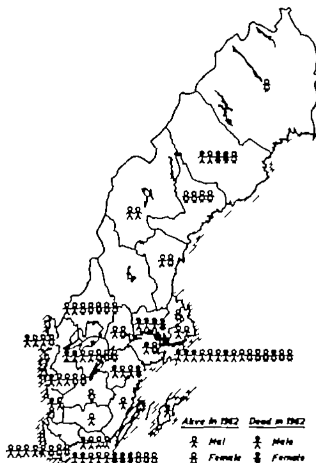


Fig. 2.

Medico-social Aspects

Little attention has so far been given to the medico-social aspects of cystinuria. According to Niemann (1876) and Link (1912) cystinuria occurs in all social classes.

As regards the geographical distribution of the cases traced in Sweden (Fig. 2) it seems that the data given in this paper reflect the intensity with which the search for cystinurics was made in the different Counties rather than the true distribution. Further large-scale screening examinations are probably required to determine the true incidence of cystinuria in the different parts of Sweden.

The civil status of the cystinurics in this series did not differ from that of a random population. Cystinuria did not affect the patients' fertility, only a few of the married patients being childless.

Information about the patients' fitness for military service was available in 20 cases. In the remaining male cases it was difficult to investigate this point, firstly because the relevant information was lacking, and secondly because the criteria for

fitness for military service used in the years from 1870 to 1960 varied greatly

Only 4 of these 20 patients had been exempt from military service on account of their cystinuria. Of the 16 patients who had been declared fit for military service, 5 developed their initial urinary tract symptoms during the first period of this service. This suggests that military service increases the danger of lithiasis. From a clinical point of view and in the light of National Health Insurance every cystinuric ought to be exempt from active military service.

Cystinuria did not appear to have determined the patients' choice of occupation. The series contains both manual labourers and white collar workers. It is interesting to note that one of the young cystinurics who was one of the most seriously ill among the patients in this series, was one of the most active amateur jockeys in Sweden at the time of this investigation. It was not possible to determine what effect this sport had on his cystinuria. Another male cystinuric, aged 22, who still played tennis when this work was in progress, had at the age of 13 developed haematuria following a tennis match.

Of the 54 male cystinurics 5 were alcoholics. This comparatively large number might have been due to the fact that these patients were unable to endure the mental strain caused by their severe renal colic and therefore became addicted to alcohol.

The average length of time for which the cystinurics in this series had received sickness benefit, suggested that cystinuria *per se* may involve medico-social problems. If the true incidence of cystinuria is the same as that revealed by the screening examination carried out by Boström & Tottie (1959) and if the prognosis for cystinurics discovered at screening examinations is as unfavourable as that in the primary and secondary cases in this series, cystinuria will no doubt be a heavier burden on the national economy than has hitherto been assumed.

Genetic Aspects

It has long been known that cystinuria is a familial disease, Marcet (1817) and Civiale (1838) each observed cases in which the patients were siblings and they therefore believed that the disease was hereditary. Since then many cases have been reported in which the cystinuria was familial. On the basis of the observations made in the case of an infant, aged two years, Niemann (1876) expressed the view that cystinuria was a congenital disease. However the elucidation of the genetics of cystinuria has presented difficulties. Dent & Harris (1951) confirmed Garrod's (1908, 1909 1923) observations, which actually are discordant, that the frequency of parental consanguinity and of cases among siblings indicated that cystinuria was a homozygous recessive disease, but the presence of affected individuals in three successive generations of the same family almost certainly indicates that some patients in this family were heterozygotes, *i.e.* the condition is sometimes dominant.

Dent & Harris (1951) could not draw any definite conclusions as to the genetics of cystinuria on the basis of eleven family trees of cystinurics published in the literature, the principle reason being that specimens of urine from the relatives of these patients were not chemically analysed. Nor did they find out more about this problem by studying the family trees of 7 of their own patients in whose families at

least one case of cystinuria had occurred. According to Harris & Warren (1953) the explanation was that no quantitative studies on urinary cystine in the relatives of these patients were carried out, only Brand's test combined with two-dimensional paper chromatography being used. Semi-cystinurics may therefore have been overlooked in a good many cases as Brand's test does not give a positive reaction if the urinary excretion of cystine is only slightly increased. This probably also explains the small number of cases of semi-cystinuria in this series, as Brand's test combined with two-dimensional paper chromatography and paper electrophoresis were the only methods used in the analyses of specimens of urine from the relatives of the patients in this series.

A further intensive search for new cystinurics and further investigation of the families in Sweden in which cystinuria has been found to occur using ion exchange chromatography for quantitative studies of the amino acids, a procedure which permits the demonstration of other amino acids as well as cystine, will probably contribute to our knowledge of the genetics of the disease

CONCLUSIONS

The results of the present investigation permit the following conclusions to be drawn:

(1) The characteristic symptoms of classical cystinuria arise from the presence of urinary calculi renal ache or the spontaneous passage of calculi being the presenting symptoms in the majority of cases.

(2) Urinary tract infection (pyelitis, pyelocystitis, cystitis) plays a more important part in cystinuria than was previously believed, and may be the cause of the initial symptoms.

(3) The incidence of urinary tract symptoms is much higher than has hitherto been assumed in cases of cystinuria.

(4) Cystinurics may form urinary calculi at any age

(5) The disease is severer in character in males than in females.

(6) Surgical treatment is required and has to be repeated in a comparatively large number of cases, particularly in male patients.

(7) Nephrectomy does not seem to have any therapeutic effect on cystinuria and should not be carried out in such cases unless there are definite indications.

(8) Cystinurics usually form more or less pure cystine stones, but may occasionally develop cystine-free stones in addition.

(9) Cystine stones are radio-opaque.

(10) Histological evidence of pyelonephritis and hydronephrosis appears to be quite common.

(11) Cystinurics are prone to develop gastro-duodenal ulceration.

(12) The prognosis for cystinuria is worse than has hitherto been assumed.

(13) The average length of time for which the cystinurics in this series received sickness benefit, suggests that cystinuria may involve medico-social problems.

(14) There is a need for improved diagnostic methods and better prophylactic measures.

(15) The routine examination of patients with urinary tract symptoms, particularly where there is a history of recurrent lithiasis or a family history of urinary tract symptoms and/or lithiasis, should always include chemical urinalysis to detect an increased excretion of cystine.

SUMMARY

This investigation followed up the studies on cystinuria which Mörner had carried out in Sweden in the years from 1922 to 1936, the cases of homozygous cystinuria traced in Sweden in the years from 1870 to 196 being discussed.

The series comprised 98 cystinurics (57 male and 41 female) who were individuals from 59 families. Urinary specimens from 1,503 members of these 59 families were analysed for homozygous cystinuria. Twenty six of these patients (17 male and 9 female) had died before this investigation was completed.

The cases were divided into the following 3 groups. (1) Primarily diagnosed cases (primary cases) (2) secondarily diagnosed cases (secondary cases) and (3) screening cases. The *primary cases* comprised 56 patients (39 male and 17 female) in whom the diagnosis of cystinuria was made when they developed urinary tract symptoms, and was based on chemical analysis of urinary calculi or on urinalysis. The *secondary cases* were 38 individuals (17 male and 21 female) who were relatives of the above 56 patients and from whom specimens of urine were collected and cystinuria was recognised. The *screening cases* consisted of 4 patients in whom the diagnosis of cystinuria was made at the screening examination of a random population of school children in Stockholm.

In 50 cases (27 males and 23 females) the patient was followed up for 40 years or more.

In 54 cases the diagnosis of cystinuria was made from qualitative and quantitative analysis of urinary calculi and urinalysis.

In 36 cases urinalysis alone was carried out, using Brand's test combined with paper chromatography and paper electrophoresis in 33 cases and Mörner's method in 3. In 5 of the 98 patients the diagnosis was made from qualitative and quantitative analysis of urinary calculi and in 3 from the patient's previous history.

Eighty-seven patients had a history of urinary tract symptoms and lithiasis: 11 had never formed stones. In 91% of the cases the patient had had urinary tract symptoms before the age of 60.

In the majority of patients the initial symptoms appeared before the age of 40. In 22 patients (16 male and 6 female) 21 of whom were primary cases, the presenting symptoms appeared before the age of 20.

In 57 cases (37 males and 20 females) renal ache or an attack of renal colic was the presenting symptom, and in 21 cases (11 males and 10 females) the initial symptoms were caused by a urinary tract infection.

Attacks of renal colic occurred in 73% of the 98 patients, and 61% passed urinary calculi spontaneously. Frequency of micturition occurred in 46%, dysuria in 38% and occasional anuria in 17% of the cases.

Macroscopic haematuria was observed in 38% of the cases, and microscopic haematuria alone in 39%. In 40% the serum N.P.N. or creatinine was increased. In 48%

there was bacteriuria, and in 4 % albuminuria.

Chemical analysis and roentgen crystallography of 143 urinary calculi from 57 cystinuric patients revealed pure cystine stones in the majority of cases. In 10 cases 13 cystine-free stones occurred in addition to cystine stones. None of the stone formers in this series passed solely cystine-free stones.

Urography revealed delayed excretion of opaque medium unilaterally or bilaterally in 26 cases (18 males and 8 females) and non-function of one kidney in 21 cases (15 males and 6 females)

In 17 cases there was radiographic evidence of dilatation of both the renal pelvis and ureter in 27 cases of dilatation of the renal pelvis only and in 7 dilatation of the ureter only In 17 cases one kidney was shown to be enlarged.

With one exception microscopic examination of the available kidney operation or autopsy specimens revealed the histological picture of pyelonephritis.

Operations for the removal of urinary calculi were performed in 60 % of the patients, 23 % being operated upon once, and 37 % between two and eight times.

Pyelolithotomy was the commonest opera tion and was the first operation performed in the majority of the cases.

I 14 cases the patient was under 20 years of age at the time of the initial operation.

The diagnosis of cystinuria was made before the first operation in 8 cases and after the first operation in 8 cases. In _2 cases the diagnosis was made only after two to six operations.

Nephrectomy was performed in 14 cases. In 8 of these urinary tract symptoms recurred.

Twenty-six patients (17 male and 9 female) died before this investigation was completed. In 12 of these cases the immediate cause of death was given as renal failure or complications of cystinuria, the average life-span of these patients being 42.8 years. In a further 12 of these cases a disease other than renal failure was given as the immediate cause of death, the average life-span of this group of patients being 68 6 years. In the remaining _ of the 26 cases renal failure was a contributory cause of death.

Peptic ulceration or gastritis co-existed in 21 % of the male patients, and in 7 % of the female patients 5 patients were alcoholics.

The cases of cystinuria in this series were distributed all over Sweden and within all occupational groups and social classes. A relationship between the type of occupation and the degree of severity of cystinuria could not be demonstrated. Only a few patients were exempt from military service on account of their cystinuria.

The patients fertility did not appear to be affected.

The capacity for work was unaffected except in 3 patients who were disabled owing to their cystinuria.

The importance of regularly following up cystinuric patients by physical examination, laboratory tests, radiography and qualitative and quantitative studies on urinary calcul and urinary cystine is emphasized.

A survey of the literature on cystinuria is given.

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APPENDIX

CASE REPORTS

Reference Numbers Used in the Case Reports

The letter M followed by the Roman figures I–XXX denotes Mörner's cases (Mörner 1922 *b* 19.6, and 193).

01 02.03 This type of reference number refers to the family trees; *the number of groups of figures* in each reference number indicates the generation in which a certain individual should be sought and *the individual figures in each group of figure* the number of steps by which this individual is removed from his ancestor. Thus the above reference number refers to the third child (in the third generation) of the second child (in the second generation) of the individual in the first generation who is the first recorded case in the family tree concerned.

Family I

Seven individuals and 5 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. I. 01. C. J. S., a shoemaker born in 1841. The patient had been healthy until the age of 25 (1856) when his first attack of renal colic occurred. This was followed by many similar attacks with occasional passage of gravel or mucus, and lasting from one hour to several days. His condition deteriorated and he lost weight, despite unimpaired appetite. At the age of 29 (1870) he developed urinary obstruction due to a calculus which blocked the urethra. The calculus was extracted. When he was 34 years old (1875) he was again admitted to hospital with chronic nephritis. He died that same year, the causes of death being given as renal calculi and nephritis. During the year preceding his death, his vision had deteriorated.

Urinalysis

1870. Considerable amounts of cystine in the specimen of urine passed following the removal of the calculus, and in 4-hr specimens passed on the following 8 days (Enwall).

Stone analysis

1870. *Urethral calculus removed that same year*, oval in shape, of yellowish-brown colour measuring 15 mm 8 mm 8 mm, weighing 0.6 g, and composed of cystine (Enwall).

1922. *Urethral calculus, removed in 1870*, composed of 97.7% cystine (ash content 0.1%), uric acid and tyrosine being absent (Mörner).

This case has earlier been reported by Enwall & Santesson in 1874 and Mörner in 1922 (M I).

DATA ON RELATIVES OF THE ABOVE CYSTINURIC

According to the account given by the patient in 1870 his mother and 3 siblings were alive at that time and suffered from the same illness as himself. As specimens of urine were not obtained from these persons, it could not be ascertained whether or not they had cystinuria.

Family II

Forty-four individuals and 5 generations were examined.

Results. 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. II. 01. A. S. W., a blacksmith, born in 1851. As a child the patient had been healthy. At the age of 33 (1884) he was kicked in the scrotum which became swollen. Following this he experienced dysuria for a few days. He had no further urinary tract symptoms until he was 49 years old (1900), when he developed backache associated with fre

quency of micturition and occasional haematuria. He was admitted to hospital and a vesical calculus *about 4 cm in diameter was* removed by *suprapubic cystolithotomy*. A year later (1901) he was again troubled by frequency of micturition associated with pain in the region of the urinary bladder. A further calculus of the same size and appearance as the previous one was removed by *suprapubic cystolithotomy*. After this operation the patient passed cloudy urine and was therefore treated by irrigating the bladder with silver nitrate solution. At the age of 54 (1905) he passed spontaneously 3 calculi, each the size f a pea. He was re-admitted to hospital and a further 12 calculi of varying size were removed *from the bladder by suprapubic cystolithotomy*. Radiography revealed a calculus of almost the size of an almond in the right kidney. This calculus was removed by *pyelolithotomy*. Following this operation he remained well for two years. Thereafter symptoms of cystopyelitis appeared. At the age f 58 (1909) a right ureteric calculus and left coral stone were removed by *ur terolithotomy* and *py lolithotomy* respectively. The following years he was cared for in hospital and another *suprapubic cystotomy* was performed, f llowed after an interval by operative closure of the ensuing bladder fistula. There was no evidence *of further stone formation. He died in* 1914 t the age of 63 the causes of death being given as cerebral haemorrhage and renal failure.

Urinalysis

Not performed

Stone analysis

1901 *Vesical calculus removed in 1900* consisting of cystine and calcium oxalate (Sjöqvist).

1921 *Vesical calculus moved in 1901* weight 16.0 g composed of cystine (82.1%), tri calcium phosphate (14.6%), ammonium magnesium phosphate (0.7%), and albumoid substance (Mörner).

1921 *Four ... cal calculi removed in 1905* weight 0.7 g, 1.3 g, 1.8 g, and 3.0 g respectively medium-sized one being analysed. It was composed of cystine (85.8%), tri calcium phosphate (9.7%), and ammonium magnesium phosphate (2.7%), calcium oxalate being *absent (Mörner)*.

1921 *Calculus removed from the left renal pelvis in 1909* coral stone, weighing 13.4 g, predominantly composed of ammonium magnesium phosphate cystine, uric acid, and oxalic acid being absent (Mörner).

This case has earlier been reported by Mörner in 1922 (M II).

Family III

Fourteen individuals and 4 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

This family could not be fully investigated because several members were unwilling to provide specimens of urine

Case No III 01 01 F L.B., an engineer born in 1886. The patient had been healthy until the age of 18 (1904) when he had his first attack of renal colic. A similar attack occurred a year later (1905), culminating in calculus blocking the urethra. The calculus had to be extracted. During the next few days he passed several small calculi spontaneously. Subsequently he was on several occasions troubled by renal symptoms associated with the spontaneous passage of calculi and had repeated ttacks of renal colic at intervals of one to three years, but he was not operated upon. He died in 1925 at the age of 39 the cause of death being given as pulmonary tuberculosis.

Urinalysis

1905 Cystine crystals in the sediment. Urinary cystine 1.5–...0 g in ...4-hr sample (Sjöqvist).

1920 Cystine crystals in the sediment; urinary cystine 1.15 g per litre (Mörner).

Stone analysis

1905 *U ethral calculus removed that same year* cystine stone (Sjöqvist).

1920 *Spontaneously passed ... lculi*. about 20 small stones, total weight 0.18 g, composed of cystine, ash content being 0.1% (Mörner).

1961 *Spontaneously passed calculus:* cystine stone (roentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by Mörner in 1922 (M III).

Family IV

Seventy-seven individuals and 6 generations were examined.

Results: 1 case of homozygous cystinuria. – cases of semi-cystinuria.

Case No IV 05 E. F. C., an engineer born in 1854. The patient had his first attack of renal colic when he was 50 years old (1904). This had been preceded by pain in the right side and occasional passage of gravel. At 5 years of age (1906) he passed a calculus. Further attacks of renal colic occurred which culminated in the passage of calculi, but he was neither X-rayed nor operated upon, and none of the stones was preserved. H died in 1923 at the age of 69 the cause of death being given as coronary arteriosclerosis.

Urinalysis

1905 A few cystine crystals in the sediment (Sjöqvist).

1920 Urinary cystine 0.05 g per litre (Mörner).

Stone analysis

No stones available.

This case has earlier been reported by Mörner in 1922 (M IV).

Family V

Three individuals and 1 generation were examined.

Results. 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No 1 02 C. D. an engineer born in 1882, whose native country was Germany but who had been resident in Sweden for several years.

The patient had been healthy until the age of 1 (1903) when the first attack of right renal colic occurred. The following year (1904) he had another attack which involved both sides and ended in the spontaneous passage of a calculus which got held up in the urethra from which it was extracted. At the age of .5 (1907) he suffered further attacks of renal colic associated with haematuria, and underwent *left ureterolithotomy* and *right pyelolithotomy*. Two years later (1909) urinary tract symptoms recurred associated with pyrexia. *Right pyelolithotomy* was again performed, the operation revealing hydronephrosis and suppurative perinephritis. The patient returned to Germany and at the age of 33 (1915) several calculi were removed by a third *right pyelolithotomy*. At the last follow-up examination in 1921 he was 39 years old and had been symptom-free during the preceding six years.

Urinalysis

1920 No cystine crystals in the sediment, urinary cystine 0.3 g per litre (Mörner).

Stone analysis

1907 *Calculus removed from the left ureter that same year* weight 40.7 g; size 93 mm .3 mm .3 mm. Cystine stone (Jolin).

1907 *Calculi removed from the right renal pelvis that same year* one weighing 22.. g and about 70 small calculi their total weight being 7–8 g; yellowish-brown stones with a finely nodular surface, composed of cystine. Specific gravity 1.590–1.689 (Jolin).

1970 *The same calculi as above:* cystine stones, ash content being 0.03–0.09% (Mörner).

1970 *Calculus removed from the right renal pelvis in 1915* cystine stone, ash content being 0..% (Mörner).

1961 *Calculus removed from the right renal pelvis in 1915* cystine stone (roentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by Floderus in 1910 and by Mörner in 192.. (M V).

Family VI

Seventy-six individuals and 5 generations were examined.

Results: 3 cases of homozygous cystinuria. No case of semi-cystinuria.

Cf. with text.

Case No VI. 01 02 E. V B., a house-wife, born in 1875 The patient was married and childless. As a child the patient had rickets and could not walk until the age of 3 (1878). She suffered from rheumatoid arthritis all her life. She had no urinary tract symptoms until the age of 37 (1912) when the first attack of renal colic occurred and cystine crystals were found in the urine. Further attacks of renal colic, with occasional passage of gravel oc curred. She passed a calculus for the first time at the age of 47 (1922). Radiography carried out seven years later (1929) did not reveal any urinary calculi. When she was 49 years old (1924) she developed gall-bladder trouble for which she was treated in hospital on several occasions. From the age of 6_ (1937) she had cardiac symptoms. At the age of 69 (1944) she was operated upon for car cinoma of the left breast. Five years later (1949) she developed bronchitis and dyspnoea. Radiography showed metastases in the mediastinum. She was given radiotherapy but ran a downward course and died in 1950 at the age of 75 the cause of death being given as carcinoma of the breast with metastases in the oesophagus and lungs.

Urinalysis

1912. Cystine crystals in the sediment (v Holst).

1920 Scanty cystine crystals in the sediment; urinary cystine 0 65 g per litre (Mörner).

1937 A few cystine crystals per high power field in the sediment (Laboratory at the Hospital in Skellefteå).

Stone analysis

1924 *Spontaneously passed stone* cystine stone ash content 0 15% (Mörner)

1961 *Spontaneously passed stone* cystine stone (roentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by von Holst in 1912 and Mörner in 1922 (M VI).

Case No VI 01 04 B. A. H., farmer born in 1877 At the age of 66 (1943) he had attacks of renal colic, there being no other urinary tract symptoms. Urography carried out in 1953 revealed right ureterolithiasis. He re mained well until the age of 74 (1951) when he was admitted to hospital with chronic cholecystitis and underwent cholecystectomy He died in 1954 at the age of 77 the causes of death being given as arteriosclerosis and cardiac failure (ascites).

Urinalysis

1920. No cystine crystals in the sediment; urinary cystine 0.36 g per litre (Mörner).

Stone analysis

N stones available.

This case has earlier been reported by Mör ner in 1922 (M IX)

Case No VI 01 07 M S., a house-wife, born in 1884 The patient was married and had nine children. Apart from cystitis on two occasions the patient remained healthy She died in 1950 at the age of 66, the causes of death being given as chronic myocarditis and cardiac failure

Urinalysis

1921 Cystine crystals in the sediment; urinary cystine 0 49 g per litre (Mörner).

Stone analysis

No stones available.

This case has earlier been reported by Mörner in 1922 (M X).

D TA ON RELATIVES OF THE ABOVE CYSTINURICS

VI 01 A male patient, who was born in 1847 and died in 1933 He had epeated at tacks of renal colic culminating in the spontaneous passage of calculi, the size of hemp seeds, which were not analysed.

Urinalysis carried out in 1912 (v Holst) and 19_2 (Mörner) did not disclose any cystine crystals in the urine.

VI. 01 04 01 A male patient, who was born in 1900 Since about 1920 there had been albuminuria and the patient had suffered from recurrent upper respiratory tract infections. When he was 24 years old (1924), he developed pneumonia and died that same year the causes of death being given as bilateral pneumonia,

left emphysema, and chronic nephritis. Post mortem examination revealed "large, pale kidneys, about twice the normal size showing a swollen, friable cortex.

Family VII

Twenty five individuals and 4 generations were examined.

Results: 1 case of homozygous cystinuria. N cases of semi-cystinuria.

Case No VII 01 C. L. B. M., a businessman, born in 1847. The patient had been healthy until the age of 64 (1911) when urinary tract symptoms in the form of frequency of micturition and dysuria appeared. Radiography performed five years later (1916) revealed a calculus in the bladder from which it was removed by *suprapubic cystolithotomy*. After the operation he had no further urinary tract symptoms. H died in 1922 at the age of 75 the cause of death being given as carcinoma of the stomach with hepatic metastases, and also arteriosclerosis.

Urinalysis

1920: No cystine crystals in the sediment, specific gravity 1.016–1.020, acid reaction (Mörner).

1921 Urinary cystine 0.02–0.03 g per litre (Mörner)

Stone analysis

1920: *Vesical stone removed in 1916* weight 25.2 g, yellowish-nodular surface, three portions of the stone were analysed.

	Calcium %	Calcium phosphate %	Substance insoluble in ammonia %	Cystine %
Peripheral portions	0.17	0.44	09	97.91
Central portions	3.55	14 31	16.90	83 10
Intermediate portions	7 41	19 14	22.99	77.01

(Mörner)

This case has earlier been reported by Mörner in 1921 and 1922 (M VII).

Cf. with text.

Family VIII

Forty-eight individuals and 5 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No VIII 01 03-01 S. A., a porter born in 1917. At the age of 18 months the patient had pneumonia and pertussis. Subsequently he enjoyed good health until he was 3 years old (1920) when he developed abdominal pain associated with anuria. A calculus was palpable in the distal urethra. It was extracted. He remained well thereafter until the age of 7 (1924) when abdominal pain associated with frequency of micturition and haematuria occurred. Radiography revealed a calculus in the right ureter. Within two weeks of the onset of the above symptoms he passed a calculus, the size of a pea spontaneously. This was followed by the passage of 4 other small calculi. Radiography performed later that same year revealed a calculus in the bladder from which it was removed by *suprapubic cystolithotomy*. In 1927 he underwent appendicectomy. At 20 years of age (1937) he again became acutely ill with right abdominal pain and ran high temperature. The urine was positive for albumen but negative for sugar. Microscopy of the urinary sediment revealed a large number of R.B.C. and W.B.C. and bacteria (cocci) per high power field. Serum N.P.N. was 39 mg%. Radiography revealed calculi in the right renal pelvis. *Nephrectomy* was performed. The kidney was found to be considerably enlarged bluish-red, oedematous, and with signs of mild perinephritis. A large number of calculi, and thick greenish pus containing Gram-positive cocci, were present in the renal pelvis.

At the age of 41 (1958) diabetes mellitus and urinary tract infection were diagnosed. The latter was resistant to sulpha drug therapy. Radiography performed year later (1959) revealed calcification of a cavity the size of hazel-nut, in the left kidney. Creatinine clearance was 156 ml per minute. B.P. was 150/90 mmHg. Three months later he developed severe cystitis. Microscopy of the urinary sediment revealed a fairly large num

ber of W.B.C. and a large number of R.B.C. per high power field. The patient attended follow-up examinations regularly and was treated with sulpha drugs. The following year (1960) he had a severe attack of left renal colic. B.P. was 150/90 mmHg. Serum creatinine was 1.4 mg%.

At the age of 42 (1959) the patient was treated in hospital for chronic alcoholism. After his discharge he was regularly followed up as an out-patient. The sequelae of a fracture of the right leg which he had sustained when he was 40 years old, his diabetes, and his renal disease compelled him to train for a lighter job. The patient had received sickness benefit for several years.

Urinalysis

1920: A large number of cystine crystals in the sediment: urinary cystine 0.61 g per litre (Mörner).

1957. Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

1920: *Urethral calculus removed that same year*: weight 0.2 g, cystine stone (Mörner).

1924. *Vesical calculus removed that same year*: cystine stone (E. Hammarsten).

1924. *Same vesical calculus as above*: cystine stone, ash content 1.22% consisting predominantly of tri calcium phosphate and traces of magnesium (Mörner).

1937. *Calculus removed from the right renal pelvis that same year*: cystine stone (Wassén).

This case has earlier been reported by Mörner in 1922 (M VIII).

Family IX

Forty-nine individuals and 4 generations were examined.

Results. 2 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No. IX 02.07. E.N., a female patient, single, born in 1881. The patient attended hospital for the first time at the age of 40 (1921) on account of attacks of renal colic which she said she had been having for several years. The previous year she had passed a calculus spontaneously. Radiography revealed a coral stone in the left kidney: this together with 6 small stones was removed by *pyelolithotomy*. She was regularly followed up on account of high blood pressure (systolic more than 180 mmHg) but otherwise remained well.

At the age of 62 (1949) the patient had a cerebral haemorrhage with resultant right hemiplegia. Six years later (1949) she had right bronchopneumonia which was treated with penicillin. After a further two years (1951) she developed cysto-pyelonephritis which cleared up following treatment with antibiotics. At 71 years of age (1952) she was admitted to hospital with uraemia. The urine was positive for albumen but negative for sugar. Microscopy of the urinary sediment revealed a large number of W.B.C. per high power field. Serum N.P.N. was 95 mg%. 300 c.c. of whole blood was transfused, and this resulted in a temporary improvement in her condition. However the serum N.P.N. level increased and she ran a downward course and died that same year, the cause of death being given as uraemia. At post-mortem examination a coral stone was found in the left kidney: it almost filled the renal pelvis: only a thin rim of the renal parenchyma remained. The parenchyma of the right kidney was virtually normal. The liver showed signs of fatty degeneration and early cirrhosis.

Urinalysis

1921. Cystine crystals in the sediment (Ramvig, Copenhagen).

1921. Scanty cystine crystals in the sediment: urinary cystine 0.46 g per litre (Mörner).

Stone analysis

1921. *Calculi removed from the left renal pelvis that same year*: coral stone 75 mm long, weight 20.4 g. Finely nodular surface of light yellowish-brown colour: cystine stone, no ash content. Additional 6 small stones: ash content 0.1% (Mörner).

1961. *Calculus removed from the left renal pelvis in 1921*: cystine stone (roentgen crystallography: Hambraeus & Lagergren).

This case has earlier been reported by Mörner in 1922 (M XI).

Case No. IX 01 06. J S.G N an agronomist, born in 1909 The patient had been healthy until the age of 6 (1935) when he had his first attack of renal colic, involving the left side. Eighteen months later a further attack of renal colic associated with haematuria occurred. Radiography revealed dilatation of the left renal pelvis, but there was no radiographic evidence of urinary calculi. Later on he passed a calculus spontaneously but it was not preserved. At the ge of 30 (1939) a further attack of renal colic occurred, ending in the spontaneous passage of gravel. There after he was symptom-free.

In 1942 the patient was operated on for perforated gastric ulcer and in 1954 for bleeding gastric ulcer

At the follow-up examination in May 1960 he was in good general condition, there being no urinary tract symptoms. B.P was 150/80 mm Hg.

Urinalysis

1957 and 1960 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

Not performed.

Family X

Twenty-nine individuals and 3 generations were examined.

Results 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case N X 06 W K.L., house-wife, born in 1864 The patient was married and had five children. She had had pneumonia on two occasions and several episodes of sore throat and rheumatic symptoms but had no urinary tract symptoms prior to the ge of 53 (1917) when she had several attacks of renal colic, culminating in the spontaneous passage of calculi. She was instructed to drink mineral water but this had no noteworthy effect and she continued to pass small calculi spontaneously at regular intervals. She was not examined radiographically nor was she operated upon. The patient died in 1949 at the age of 85 the cause of death being given as "decrepitude"

Urinalysis

1922. Cystine crystals in the sediment, urinary cystine 0.19–0.25 g per litre (Mörner).

Stone analysis

1922. *Spontaneously passed calculus:* cystine stone, ash content being 0.05% (Mörner).

1961 *Spontaneously passed calculus:* cystine stone (roentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by Mörner in 1922 (M XII).

D TA ON RELATIVES OF THE ABOVE CYSTINURIC

Four of the patient's (X 06) siblings (X 02–05) died in infancy the cause of death being unknown.

Family XI

Two individuals and generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No XI 01 P P timber-merchant, born in 1860 The patient had been healthy until the age of 44 (1904) when he had an attack of bilateral renal colic. Thereafter he remained well until the age of 61 (1921) when further attack of renal colic on the right side occurred, ending in the spontaneous passage of calculi. Radiography carried out the following year (1922), revealed calculi in the left kidney The patient was not operated upon and died in 1946 at the age of 86, the cause of death being given as decrepitude

Urinalysis

1923 Cystine crystals in the sediment; urinary cystine 1 15 g per litre (Morner).

Stone analysis

1922. *Spontaneously passed calculus* cystine stone (Widmark).

1923 *Spontaneously passed calculi* 0 stones, total weight 0.52 g, composed of cystine and tri calcium phosphate ash content being 0.04% (Mörner).

1961 *Spontaneously passed calculus*: cystine stone (roentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by Mor ner in 1926 (M XIII).

Data on Relatives of the Above Cystinuric

According to the information obtained the patient' (XI 01) mother had renal ache between the ages of 45 and 50 and died at the age of 67 the cause of death being given as pneumonia. The patient's father had not had any urinary tract symptoms. He died at the age of 76, the cause of death being given as coronary arteriosclerosis. One of the patient s daughters (XI. 01 04) was still alive at the time of this investigation and had never had any urinary tract symptoms. With her death the patient's family becomes extinct.

Family XII

Nineteen individuals and 4 generations were examined.

Results: 2 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No XII 01 02 E. M. M a housewife born in 1880. The patient was married and had three children. At the age of 30 she had her first attack of renal colic culminating in the spontaneous passage of calculus which was not preserved. At the age of 58 (1938) a similar ttack occurred. At the age of 6.. (194) she developed cystitis on two occasions, there being no other urinary tract symptoms. At the age of 75 (1955) she developed hyper tensive symptoms, and had several minor myocardial infarctions. From 1958 to 1959 she was in hospital with myocardial infarc tion. During her stay in hospital serum N P.N was between 31 mg% and 60 mg% B P varied between 230 mmHg and 250 mmHg systolic and 140 mmHg and 145 mmHg diastolic. The patient died in 1959 at the age of 79 the cause of death being given as coronary arteriosclerosis.

Urinalysis

1923 Cystine crystals in the sediment; urinary cystine 0.74 g per litre (Mörner).

1957 Abnormal amounts of cystine lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

Not performed.

This case has earlier been reported by Mör ner in 1926 (M XV).

Case No XII 01 07 E. E. L., a printer born in 1890. From the age of 26 (1916) he had had several attacks of left renal colic which were occasionally associated with ma croscopic haematuria. At the age of 31 (1921) he passed a calculus which got held up in the urethra, from which it had to be extracted. Two years later (1923) he passed several cal culi spontaneously Radiography revealed the presence of two calculi, one in the left kidney and the other in the bladder These calculi were removed by *pyelolithotomy* and *suprapubic cystolithotomy* respectively Thereafter he was symptom-free until the age of 44 (1934) when a further attack f left renal colic occurred. B P was at that time 140 105 mmHg. Radiography revealed a coral stone in the left renal pelvis. *Left nephrectomy* was performed. Mac oscopically the operation specimen showed hydronephrosis and signs of chronic pyelonephritis. Post-operatively serum N.P.N. rose to 70 mg% A further attack of renal colic on the other side occurred and was associated with anuria. *S prapubic cystotomy* and *right ureterotomy* were performed. The calculus was lodged in the distal part of the ureter and proved to be irremovable, but fortunately the patient did not have any further urinary tract symptoms At the age of 30 (1920) he was re admitted to hospital with suspected gastric ulceration and at the age of 31 (1921) with chronic gastroenteritis. Thereafter he had several attack of lumbago and sciatica. At the age of 60 (1950) intermittent claudication ppeared which became progressively worse despite medical and surgical treatment. At the

ge of 63 (1953) he developed mild diabetes. At 65 (1955) he had a myocardial infarction. Three years later (1958) he was re-admitted to hospital with second myocardial infarc tion. Serum N.P.N. was then 52 mg%. H recovered from this attack but died four months later at the age of 68 (1958), the cause of death being given as myocardial infarction.

Urinalysis

1923. Cystine crystals in the sediment urinary cystine 0.6 g per litre (Mörner).

1957. Abnormal amounts of cystine lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1924. *Vesical calculus removed in 1923* weight 0.6 g, cystine stone, ash content being 7.62% the ash consisting of tri calcium phosphate and small amount of magnesium phosphate (Mörner).

Calculus removed from the left renal pelvis in 1923 weight 5 1 g, cystine stone, ash content being 2.46% the ash consisting of tri calcium phosphate and small amount of ammonium magnesium phosphate (Mörner)

1935. *Calculus emoved from the left renal pelvis that same year* coral stone composed of tri calcium phosphate, ammonium magne sium phosphate and calcium carbonate, cystine being absent (Blix).

This case has earlier been reported by Mor ner in 1926 (M XIV).

Family XIII

Fifty individuals and 5 generations were examined.

Results. 1 case of homozygous cystinuria. No case of semi-cystinuria

Case N XIII 01 01 01 J P road la bourer born in 1899 At the ge of 15 (1914) he had albuminuria. At the age of 20 (1919) he had dysuria, frequency of micturition, and passed cloudy urine. Radiography performed four years later (1923) revealed a large cal culus in the bladder it was removed by *suprapubic cystolithotomy* Thereafter he remained well.

At the follow-up examination in June 1960 he was in good general condition, there being no urinary tract symptoms. B.P was 140/80 mmHg.

Urinalysis

1923. Cystine crystals in the sediment urinary cystine 0.42 g per litre (Mörner).

1957 and 1960. Abnormal amounts of cystine lysine arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1923. *Vesical calculus removed that same year* weight 50.2 g, measuring 51 mm × 41 mm 29 mm, and composed f cystine of a high degree of purity ash content being between 0.23% and 0.61% the ash consisting of tri calcium phosphate the purest cystine being found in the central parts of the stone (Mörner).

This case has earlier been reported by Mör ner in 1926 (M XVI) and Hambraeus & Boström in 1960.

Family XIV

Forty-one individuals nd 4 generations were examined.

R sults. 2 cases of homozygous cystinuria, 6 cases of semi-cystinuria.

Case N XIV 01 01 A.W J B., labourer born in 1905 At the age of 18 months (1907) a calculus was removed from the bladder by *suprapubic cystolithotomy* At the age of 17 (1922) he developed haematuria following a ride in a jolting cart Radiography revealed the presence of calculus in the bladder it was removed by *suprapubic cystolithotomy* A year later (1923) he developed pain in the region of the right kidney which was associated with dysuria and frequency of micturition. There was no evidence f calculi but there was urinary infection which was treated with hexamethylenetetramine. Later on there was radiographic evidence f calculus in the right kidney The urine was found to be alkaline and negative for albumen and sugar Microscopy of the urinary sediment revealed numerous W.B.C and a large number of

Gram-positive bacteria per high power field. In 1924 *right pyelolithotomy* was performed, the operation revealing marked perinephritis; a slightly flattened calculus, the size of a hazelnut, was removed from the renal pelvis. No further data on the patient' condition were available. He died in 1929 at the age of 24 the cause of death being given as renal failure.

Urinalysis

1924 Cystine crystals in the sediment; urinary cystine 0 76 g per litre (Mörner).

Stone analysis

1924 *Calculus removed from the right renal pelvis that same year* weight 2.0 g, composed of cystine (Blom).

1924 *The same calculus as above:* cystine stone, ash content being 0.02% (Mörner).

1926. *Vesical stone removed in 1922.* cystine stone, ash content being 0.2% (Mörner).

1961 *Vesical calculus removed in 1922* cystine stone (roentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by Mörner in 1926 (M XVII).

Case No. XIV 01-02 M. G. V N housewife, born in 1908. The patient was married and had two children. As a child she had experienced dysuria and had occasionally passed gravel spontaneously Radiography (1934) revealed hydronephrosis on the right side, but there was no evidence of the presence of urinary calculi. In the following year the patient was admitted to hospital with an attack of renal colic. There was no albuminuria. Microscopy of the urinary sediment revealed numerous R.B.C. and W.B.C. per high power field. Bacteria were absent. A few days after admission she passed a urinary calculus twice the size of a grain of rice. Retrograde pyelography revealed mild hydronephrosis on the right side. Thereafter she remained well for four years, being re-admitted to hospital in 1939 with an attack of right renal colic which ended in the spontaneous passage of a calculus. Urography did not reveal any abnormality Thereafter she again remained well until the age of 41 (1949) when she had an attack of left renal colic. There was no albuminuria. Microscopy of the urinary sediment revealed between eight and ten W.B.C. and numerous R.B.C. per high power field. Urography did not reveal any abnormality The calculi were not preserved.

At the age of 26 (1934) the patient developed duodenal ulceration which was treated medically When she was 36 (1944) her duodenal ulcer symptoms recurred and partial gastrectomy was performed. Post-operatively she developed paralytic ileus and enteroanastomosis was done.

At the follow-up examination in May 1960 she showed impairment of hearing, but was otherwise in good condition. She had not had any further urinary tract symptoms.

Urinalysis

1924 Cystine crystals in the sediment, urinary cystine 1.04 g per litre (Mörner).

1957 and 1960· Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

Not performed.

This case has earlier been reported by Mörner in 1926 (M XVIII).

D T ON A RELATIVE OF THE ABOVE CYSTINURICS

No. XIV 08. This was the case of the mother of the 2 cystinurics traced in this family She had had urinary tract symptoms but not cystinuria, according to Mörner

Family XV

Forty-three individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. N case of semi-cystinuria.

Case No. XV 01 01 01 H. A. N a businessman, born in 1911 At the age of 11 (1922) the patient had several attacks of bdominal pain of unknown cause. Six months later he developed pneumonia. While being treated for the pneumonia frequency of micturition was noted. He eventually developed pain in the region of the urinary bladder. Vesical calculi

were palpated. In 1923 3 calculi were removed from the bladder by *suprapubic cystolithotomy* At the age of 13 (1924) he had two attacks of renal colic, each culminating in the spontaneous passage of calculi. The following year (1925) he had an attack of right renal colic associated with haematuria. Pain in the region of the bladder recurred, and at the age of 15 (1926) he underwent a second *suprapubic cystolithotomy* Thereafter further mild attacks of renal colic occurred. At the age of 20 (1931) there was an attack of renal colic followed by the spontaneous passage of a calculus. Radiography performed that same year revealed a calculus in either kidney that in the right being a coral stone. When he was 21 (1932) a calculus got held up in the left ureter from which it was removed by *ureterolithotomy* A year later (1933) serum N.P.N was 32 mg% Thereafter he had several episodes of pyelitis and passed calculi spontaneously

At the age of 35 (1946) he developed a bleeding gastric ulcer which was treated medically At the age of 42 (1953) he developed goitre and year later (1954) hypertension (B.P being 230/120 mmHg) associated with cardiac symptoms and mild uraemia (serum N.P.N being between 55 mg% and 66 mg%) Cardiac and renal function became progressively worse and there was anuria which was relieved by catheterisation. The patient ran downward course and died at the age of 43 in 1954 the causes f death being given as chronic glomerulonephritis and nephrolithiasis.

Urinalysis

1925 No cystine crystals in the sediment; very alkaline urine: urinary cystine 0.79 g per litre (Mörner).

1932. A fairly large number of cystine crystals in the sediment (Laboratory at the Hospital in Borås).

Stone analysis

1925 *Vesical calculi removed in 1923* 3 stones, weighing 35.7 g, 2.6 g, and 1.5 g respectively composed of cystine, the ash content of the largest stone being 0.51% that of the 2 smaller ones 0.42% The ash consists of tri calcium phosphate (Mörner).

1926. *Vesical calculus removed that same year* phosphate stone weight 6.0 g, cystine being bsent (Mörner).

1931 *Calculus passed spontaneously* cystine stone, ash content being 2.7% (Mörner).

1961 *V sical stone removed in 1923* cystine stone (roentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by Mörner in 1926 (M XIX).

Family XVI

One hundred and seventy-one individuals and 5 generations were examined.

Results 3 cases of homozygous cystinuria. 11 cases of semi-cystinuria.

Case No. XVI 01·06 02 J V K., a farmer born in 1895 At the age of 21 (1916) the patient developed left renal ache which persisted for 5 days. At the age of .3 (1918) his urine was positive for albumen. Three years later (1921) he had an attack of renal colic which ended in the spontaneous passage of calculus and was associated with haematuria. At the age of 33 (1928) he was admitted to hospital with rupture of the left kidney following an accident. Since 1921 he had passed calculi spontaneously every year without experiencing any noteworthy discomfort. At the age of 52 (1947) he was admitted to hospital after having had tw further attacks of renal colic which were severe in character and culminated in the spontaneous passage of calculi. His urine showed trace f albumen and was negative for sugar Microscopy of the urinary sediment revealed between two and four R.B C. and W.B.C. per high power field. Bacteria were absent. Radiography revealed bilateral renal calculi. He was treated with alkalis. Two years later he had a further attack of severe renal colic, ending in the spontaneous passage of several calculi. At the age of 60 (1955) he developed cardiac symptoms and was admitted to hospital with hypertension (B.P 250 120 mmHg). Radiography again revealed bilateral renal calculi. The urine was positive for both albumen and sugar Microscopy of the urinary sediment revealed only few R.B.C and

W.B.C per high power field. Serum N.P.N was between 35 mg% and 61 mg% Serum creatinine was between 2.2 mg% and 2.3 mg% Creatinine clearance was 42 ml per minute. He was put on the sick-list and granted national retirement pension.

At the follow-up examination in June 1960 he showed slight cyanosis of the lips and slight dyspnoea after exertion. B.P was 250/130 mmHg.

Urinalysis

1925 Cystine crystals in the sediment, urinary cystine 0.2.. g per litre (Mörner).

1947 Abnormal amount of cystine (Laboratory at the Hospital in Umeå).

1956 and 1960: Abnormal amounts of cystine, lysine arginine and ornithine (Boström & Hambraeus).

Stone analysis

1947 *Urinary calculus passed spontaneously* cystine stone (Jungner).

1961 *Urinary calculi passed spontaneously* 2 cystine stones (oentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by Mörner in 1926 (M XXII).

Case No XVI 01 06 05 A. E. K., a head master born in 1903 The patient had been healthy until the age of 18 (1921) when he had his first attack of left renal colic. Microscopy of the urinary sediment revealed large number of R.B.C and W.B.C per high power field. Further attacks of renal colic involving both sides nd culminating in the spontaneous passage of calculi occurred. Radiography (1925) evealed bilateral renal calculi, the largest calculi being on the right side Serum N.P.N was 34 mg% B.P was 100 mmHg systolic. *Right pyelolithotomy* and *ureterolithotomy* were performed. A calculus, measuring 4.0 cm 1.5 cm, was removed f om the renal pelvis which was dilated the renal parenchyma was more or less normal Post-operatively the patient had an attack of pain in the left side, ending in the spontaneous passage of calculi. During the next three years he had no noteworthy urinary tract symptoms In 1928 there were further attacks of left renal colic which ended in the spontaneous passage of calculi and were associated with haematuria. The following year (1929) he was admitted to hospital. The urine was negative for albumen and sugar Microscopy of the urinary sediment revealed a large number of R.B.C. per high power field. B.P was 140 mm Hg systolic. Radiography evealed calculi in the left renal pelvis and *pyelolithotomy* was performed. The operation revealed moderate perinephritis. Three calculi, one the size of a thumb nail and .. the size of peas, were removed f om the left renal pelvis. A year later (1930) there was a further attack of renal colic associated with albuminuria and ending in the spontaneous passage of calculi. Thereafter attacks of renal colic culminating in the spontaneous passage of calculi recurred. Pyelonephritis and hypertension (?00/130 mmHg) co-existed. Serum N P.N was within normal limits (<30 mg%). Microscopy of the urinary sediment revealed between ten and fifteen R.B.C a large number of W.B.C. per high power field and bacteria (staphylococci). At the age of 35 (1938) he underwent *left nephrectom* the operation revealing marked perinephritis, several large calculi in the renal pelvis, and extensive f brotic change in the renal parenchyma. Thereafter he remained well until the age of 46 (1949) when he had repeated episodes of cystitis associated with pyrexia (39.5 C)[1] and epididymitis, being admitted to hospital on account of the latter B P was 195/115 mmHg. At the age of 54 (1957) he had an attack of right renal colic associated with anuria. Serum N.P.N was 50 mg% Radiography revealed a calculus, the size of a bean, in the ureter Drainage of the kidney was effected by insertion of ureteric catheter In view of the size of the ureteric calculus *right ureterolithotomy* was thereafter performed B P was 10/110 mmHg. At the age of 50 (1953) he suddenly developed dizziness, malaise, and a feeling of numbness in the left hand. The following day there was paresis of the muscles of that hand, sensation being unaffected. On admission to hospital B.P was 210 120 mmHg but fell to 180/110 mmHg during his stay in hospital. Serum

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[1] Rectal temperature.

N.P.N. was 34 mg%. The paresis of the left hand regressed quickly.

From the age of 41 (1944) he had been suffering from duodenal ulceration in addition. Medical treatment of the ulcer was ineffective. At 52 (1955) he underwent gastroenterostomy. Post-operatively serum N.P.N. rose to 50 mg%.

At the follow-up examination in June 1960 the patient was in good general condition and had no urinary tract symptoms. B.P. was 150/90 mmHg.

Urinalysis

1925 Cystine crystals in the sediment, urinary cystine 0.44 g per litre (Mörner).

1957 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1925 *Calculus removed from the right renal pelvis:* cystine stone (Silwer).

1925 *the same calculus as above:* cystine stone, ash content 0.30% the ash being composed of tri calcium phosphate (Mörner).

1925 *Calculus passed spontaneously:* cystine stone (Mörner).

1961 *Calculus removed from the right renal pelvis in 1925:* composed predominantly of cystine, small amounts of struvite and apatite being present in addition (roentgen crystallography Hambraeus & Lagergren).

1961 *Calculus removed from the left renal pelvis in 1938:* composed predominantly of cystine, small amounts of struvite and apatite being present in addition (roentgen crystallography Hambraeus & Lagergren).

This case has earlier been reported by Mörner in 1926 (M XX).

Case No. XVI 01 06 E. S. T., a house-wife, born in 1906. The patient was married and childless. She had been healthy until the age of 25 (1931) when she had an attack of renal colic. Thereafter she remained well until the age of 49 (1955) when she had an attack of left renal colic and was operated upon (*left ureterolithotomy*). B.P. was 160/100 mmHg. Her urine contained albumen but no sugar. Serum N.P.N. was 54 mg%. She also had mild right nephritic pain and had passed stones spontaneously on several occasions.

At the follow-up examination in June 1960 her general condition was found to be good and she had no urinary tract symptoms. B.P. was 140/90 mmHg.

Urinalysis

1925 Cystine crystals in the sediment; urinary cystine 0.43–0.75 g per litre (Mörner).

1956 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1955 *Ureteric stone* measuring 10 mm × 6 mm × 4 mm, in size. Analysis was not carried out.

This case has earlier been reported by Mörner in 1926 (M XXI).

Data on a Relative of the Above Cystinurics

The mother of the above cystinurics was the daughter of one of their father's cousins.

Family XVII

Seventeen individuals and 3 generations were examined.

Results. 3 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No. XVII 01 01 B. E., house-wife, born in 1906. The patient was married and had two children. Between 1930 and 1950 she had had acute cystitis on several occasions. During her pregnancies her urine was positive for albumen, there being no other signs or symptoms of physical disease.

Urinalysis

1922 Cystine crystals in the sediment (Sjöqvist).

1947 Cystine crystals in the sediment (Swedin).

1957 Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

This case has earlier been reported by Mörner in 1932 (M XXIV).

Case No XVII 01 03 E. W a house-wife, born in 1910. The patient was married and had one child. She had her first attack of renal colic at the age of 13 (1922) but did not pass any stone spontaneously Seven years later (1929) another attack of renal colic occurred which culminated in the spontaneous passage of stones. At the age of 26 (1936) she had a third attack of renal colic. Radiography at that time revealed a coral stone in the right renal pelvis from which it was removed by *pyelolithotomy* Prior to the operation the urine was negative for both albumen and sugar Microscopy of the urinary sediment revealed a large number of W.B.C. and bacteria per high power field. B.P was 130/75 mmHg and serum N.P.N was 29 mg% Nine years later (1945) she had several episodes of cystopyelitis associated with pyrexia. Radiography revealed the presence of calculi in the right kidney The urine was found to be positive for albumen. Microscopy of the urinary sediment revealed between ten and twelve R.B.C., a large number of W.B.C., and bacteria per high power field. Serum N.P.N was between 32 mg% and 46 mg% *Right nephrectomy* was performed, the operative findings being as follows: The kidney was enlarged and there were marked perinephritic adhesions, there was extensive necrosis of the parenchyma, coral stone, the size of a pigeon egg, and pus were present in the renal pelvis. Post-operatively the patient developed a wound abscess which settled down. Thereafter she had no further urinary tract symptoms. At the age of 35 (1945) she underwent Caesarean section, the post-operative course being complicated by paralytic ileus.

At the follow-up examination i May 1960 her general condition was found to be good but she was ery obese. B.P was 145/95 mmHg.

Urinalysis

1922. Cystine crystals in the sediment (Sjöqvist).

1947 No cystine crystals in the sediment (Swedin).

1957 and 1960· Abnormal amounts of cystine, lysine arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1929· *Calculus passed spontaneously* cystine stone (Sjöqvist).

1936 *Calculus removed from the right renal pelvis in 1936* cystine stone with a finely nodular surface f yellowish-brown colour (Laboratory at St. Göran s Hospital, Stockholm).

This case has earlier been reported by Mörner in 1932 (M XXIII).

Case No XVII 01 06 U M T., a house wife, born in 1923 The patient was married and had had four pregnancies, the second baby being the only one of the four born alive. She had undergone appendicectomy the post-operative course being complicated by peritonitis.

At the age of 21 (1944) she had her first attack of renal colic involving the right side and ending in the spontaneous passage of a stone. On the following day a similar attack occurred involving the left side. Two years later in 1946 a third attack of renal colic occurred but, there was no spontaneous passage f stones. At the age of 24 (1947) she was admitted to hospital with right renal pain and haematuria. The urine was found to contain a trace of albumen but no sugar Microscopy of the urinary sediment revealed a few R.B.C. and numerous bacteria per high power field. Serum N.P.N was 22 mg% Radiography revealed a calculus in the right renal pelvis from which it was removed by *pyelolithotomy* Thereafter attacks of renal colic culminating in the spontaneous passage of stones recurred at oughly two yearly intervals. Between these attacks she had bilateral renal ache and cystitis on several occasions.

At the follow-up examination in 1960 her general condition was found to be good and she had no urinary tract symptoms. B.P was 140 95 mmHg.

Urinalysis

1947 Cystine crystals in the sediment (Swedin).

1957 and 1960· Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1944 *Calculi passed spontaneously* 2 calcium phosphate stones (Laboratory at Sabbatsberg's Hospital, Stockholm).

1947 *Calculus removed from the right renal pelvis that same year* cystine stone (Swedin).

1961 *Calculi passed spontaneously* 2 stones composed predominantly of cystine, a small amount of apatite being present in addition (roentgen crystallography Hambraeus & Lagergren).

Family XVIII

Two individuals and 1 generation were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No XVIII 01 D. H. M A., a workman, born in Russia in 1876. At the age of 50 (1926) the patient had his first attack of renal colic which was associated with frequency of micturition. There was no albuminuria. Cystoscopy revealed calculus which had got held up t the right ureteric orifice. Microscopy of the urinary sediment revealed between five and ten R.B.C. per high power field. Radiography did not reveal any calculi. Two years later (1928) he had another attack of renal colic ending in the spontaneous passage of a calculus the size of pea. There was no further information about the patient available. He died at the age of 60 (1936), the cause of death being given as chronic myocarditis.

Urinalysis

1926. Cystine crystals in the sediment (L. Bostrom).

Stone analysis

1928 Calculus passed spontaneously in 1928. cystine stone, uric acid, oxaluric acid, carbonic acid, ammonium and metals being absent (E. Hammarsten).

This case has earlier been reported by Mörner in 193 (M XXV).

Dat on Relative of the Above Cystinuric

XVIII. 02. A female patient, born in 1882. She was admitted to hospital with abdominal pain of unknown cause which might have been due to a urinary calculus. There was no history of passage of either stones or gravel.

Family XIX

Twenty-six individuals and 3 generations were examined.

Results: 4 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No. XIX 01 E. I. O cook (female), single, born in 1887 Since the age of 35 (1922) the patient had had repeated attacks of left renal colic, ending in the spontaneous passage of calculi. When she was 47 years old (1934) radiography revealed a stone in either kidney the one in the right being a coral stone. The right kidney showed radiographic evidence f caseo-calcareous tuberculosis. Microscopy of the urinary sediment revealed few R.B.C. and W.B.C. per high power field. There was no bacteriuria. On admission to hospital her serum N.P.N. was 63 mg% *Left pyelolithotomy* was performed. Post-operatively her serum N.P.N fell to 22 mg% Thereafter the patient remained well until 1949 when the urine was found to contain albumen. The serum N.P.N level wa raised and hypertension was diagnosed. She was, therefore, re-admitted t hospital. Her urine was found to contain albumen but no sugar Microscopy of the urinary sediment revealed a large number of W.B.C. and bacteria per high power field. Serum N.P.N was 70 mg% B.P was 200/110 mmHg. Radiography revealed a calculus, the size of a hen s egg, in the right kidney and several small calculi in the left. The patient went downhill, and shortly before her death at the age of 62 (1949), her serum N.P.N rose from 100 mg% to 300 mg% The cause of death was given as uraemia.

At post-mortem examination the right kidney was found to be considerably enlarged and dilated a large number of calculi and pus were present in the renal pelvis. The left kidney was adherent to surrounding tissue and contained a large number of calculi, there was only a thin rim of renal parenchyma left

in the renal pelvis was some turbid fluid which was not pus.

Urinalysis

Not performed.

Stone analysis

Not performed

The history of this patient was considered to be sufficiently characteristic to permit the diagnosis of cystinuria to be made (Hambræus, 1960).

Case No XIX 03 N O sailor born in 1894 At the age of 22 (1916) the patient noted the presence of gravel in the urine. Five years later (1921) he developed haematuria there being no other urinary tract symptoms. However bilateral renal calculi were diagnosed radiologically At the age of 28 (1922) he underwent *right py lolithotomy* a coral stone plus 85 calculi of varying size being removed. The immediate post-operative course was uneventful. Five months later however he was re-admitted to hospital with a blood pressure of 110 mmHg systolic, the serum N P.N being 62 mg% *Left pyelolithotomy* was performed, a large coral stone and 27 small calculi being removed. The operation revealed dilatation of the renal pelvis and extensive trophy of the renal parenchyma. Post-operatively serum N P N rose to 120 mg%. The patient ran a downward course and died (1922) six days after the operation, the cause of death being given as post-operative haemorrhage from the kidney

Urinalysis

Not performed.

Stone analysis

1930: *C lcul removed from the right renal pel is that sam year* a coral stone, weighing 31.9 g and several small stones, their total weight being 13 2 g, all the stones being composed of cystine, ash content being 0.1% (G Hammarsten).

1930: *C lc li removed from the left renal pelvis that same ea* a coral stone weighing 31.9 g and several small stones, their total weight being 8.3 g. ll the stones being composed of cyst ne (G Hammarsten)

This case has earlier been reported by G Hammarsten in 1931 Mörner in 1932 (M XXVI) and Widmark & G Hammarsten in 1933

Case No XIX 05 S.T O a house-wife born in 1899 The patient was married and had one child. At the age of 20 (1919) she had her first attack of right renal colic. As several further mild attacks of renal colic had occurred during the following year she was admitted to hospital. The urine was found to contai albumen but no sugar Microscopy of the urinary sediment revealed numerous R.B C., W.B.C., and bacteria per high power field Radiography revealed calculi in the right renal pelvis. *Right pyelolithotomy* was therefore performed, the operation revealing 2 cal culi the size of a walnut and some gravel. Thereafter the patient remained well for eight years. However she was re-admitted to hospital in 1928 with headache, vomiting, dimness of vision, and albuminuria. B.P was 220 mmHg systolic. The urine was found to contain albumen but no sugar Microscopy of the urinary sediment revealed numerous R.B.C. and W B.C. and fragments of hyaline casts per high power field. Serum N P.N was 120 mg% The patient ran a downward course. The serum N P.N rose to 170 mg% and she died at the age of 30 (1929), the cause of death being given as uraemia.

Urinalysis

Not performed.

Stone analysis

1920. *Calc lus removed from the right renal pelvis that sam year:* friable stone, the size of a pea, which fell into pieces when being preserved, its macroscopic appearance only being described in the hospital records.

The previous history of this patient was considered to be sufficiently characteristic to permit the diagnosis of cystinuria to be made (Hambræus, 1960).

Case No. XIX 04 01 01 G O., a female student, single, born i 1947 The patient enjoyed good health and had no urinary tract symptoms. In May 1960 her general condition

was found to be good, there being no urinary tract symptoms.

Urinalysis

1957 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

DATA ON A RELATIVE OF THE ABOVE CYSTINURICS

XIX. 06. A Custom-House employee, born in 1905. The patient was a brother of three of the cystinurics (XIX. 02, XIX. 03 and XIX. 05) traced in this family. H. had been healthy until the age of 20 (1925) when he caught a bad cold while doing his military service. Thereafter he developed asthma which made him unfit for work. Ten years later (1935) he was admitted to a nursing home with haemoptysis. At the same time he developed abdominal pain of unknown cause and died at the age of 31 (1936) the cause of death being given as uraemia. Post-mortem examination was not performed.

Family XX

Eighteen individuals and 3 generations were examined.

Results. 3 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No. XX 01 01. E. E. S., a house-wife, born in 1909. The patient was married and had three children. At the age of 10 (1919) she had osteitis of the right tibia. When she was 25 years old (1934) she developed left-sided pyelitis. Thereafter she occasionally had renal ache, frequency of micturition, and dysuria associated with haematuria. When she was 35 years old (1942) radiography revealed calculus in the bladder. There was no albuminuria, and examination of the urinary sediment revealed no abnormality. B.P. was 140/95 mmHg. *Suprapubic cystolithotomy* was performed. After the operation she was symptom-free.

At the follow-up examination in May 1960 her general condition was found to be good. B.P. was 140/90 mmHg. She had no urinary tract symptoms.

Urinalysis

1930: Cystine crystals in the sediment (Husmark).

1958 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

Not performed.

This case has earlier been reported by Morner in 193 (M XXVIII).

Case No. XX 01 04. T. F. G., carpenter, born in 1917. The patient had measles at the age of 3 (1920). Thereafter he remained in poor health for a long time. At the age of 11 (1928) his urine was found to contain albumen. Two years later (1930) there was frequency of micturition and dysuria. Radiography revealed multiple large calculi in the bladder. *Suprapubic cystolithotomy* was performed. The urine was negative for albumen. Microscopy of the urinary sediment revealed numerous W.B.C., bacteria, and few cystine crystals per high power field. After the operation the patient remained well until the age of 30 (1947) when he had an attack of right renal colic which culminated in the spontaneous passage of calculus. Six years later (1953) he developed bilateral renal ache and cystopyelitis was diagnosed. There was radiological evidence of right hydro-ureter. B.P. was 150/90 mmHg. Serum N.P.N. was 22 mg%. There was albuminuria. Microscopy of the urinary sediment revealed a few R.B.C. and ten to fifteen W.B.C. per high power field. At the age of 40 (1957) he had attacks of nausea associated with headache and vomiting, there being no urinary tract symptoms. Two years later (1959) a similar attack occurred. He was found to have albuminuria and hypertension (B.P. 215/105 mmHg) and was admitted to hospital for investigation. Radiography revealed hypoplasia of the left kidney and right hydroureter. Serum N.P.N. was between 28 mg% and 39 mg%. Serum creatinine was 1.1 mg%. Creatinine clearance was 113 ml per minute.

At the follow-up examination in May 1960

the patient's general condition was found to be good. B.P was 180/120 mmHg.

Urinalysis

1930: Cystine crystals in the sediment (Husmark).

1956 and 1960: Cystine crystals in the sediment. Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

1930: *Vesical calculi removed that same year*: 5 stones (total weight 90 g) composed of cystine, ash content varying between 0.5% and 5% (Mörner).

1961: *Calculus removed from the bladder in 1930*, composed predominantly of cystine, small amounts of struvite and apatite being present in the superficial portions in addition (roentgen crystallography: Hambraeus & Lagergren).

This case has earlier been reported by Mörner in 1930 (M XXVII).

Case No. XX 01 08. N. A. E. G., a carpenter born in 1924. When the patient was 6 years old (1930) microscopic haematuria was found in the absence of urinary tract symptoms. At the age of 8 (1932) he was operated upon for ileus. At the age of 20 (1944) he had scarlet fever complicated by nephritis. B.P was 155/100 mmHg. His urine was found to contain albumen but no sugar. Microscopy of the urinary sediment revealed between fifty and sixty R.B.C. and between ten and fifteen W.B.C. per high power field. Serum N.P.N. was between 37 mg% and 49 mg%. At the age of 21 (1945), while on active service, he had his one and only attack of renal colic. Radiography did not reveal any abnormality.

At the age of 34 (1958) the patient developed a bleeding gastric ulcer which was treated medically. B.P. was 1?5/80 mmHg. There was albuminuria. Microscopy of the urinary sediment revealed between eight and ten R.B.C. and a few W.B.C. per high power field. Bacteria were absent. Serum N.P.N. was 31 mg%.

At the follow-up examination in May 1960 the patient's general condition was found to be good. B.P. was 150/95 mmHg. He had no urinary tract symptoms.

Urinalysis

1930: Cystine crystals in the sediment (Husmark).

1956 and 1960: Cystine crystals in the sediment. Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

This case has earlier been reported by Mörner in 1932 (M XXIX).

Family XXI

Thirty-seven individuals and 4 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XXI 05. E. K. R., a nurse, born in 1888. The patient was married and had two children. During her first pregnancy at the age of 24 (1912), she developed cystitis associated with pyrexia (39 C). She had several further episodes of cystitis thereafter. Three years later (1915), during her second pregnancy, acute cystitis similar in character to that which she had had during her first pregnancy recurred. Between these severe episodes of cystitis she passed on one occasion 4 small calculi spontaneously. In 1920 tuberculosis of the right kidney was suspected and she was admitted to hospital. One year later (1921) she had an attack of right renal colic, culminating in the spontaneous passage of a calculus. Thereafter she was symptom free until the age of 4? (1930) when she had a further attack of renal colic ending in the spontaneous passage of a calculus. Two months later another attack of renal colic occurred associated with oliguria and moderate uraemia. B.P. was 160/100 mmHg. Serum N.P.N. was 68 mg%. The volume of urine voided gradually increased and serum N.P.N. returned to normal. Radiography of the kidneys did not

reveal any abnormality The following year (1931) she had yet another attack of renal colic associated with lituria. There was albuminuria. B.P was 140 mmHg systolic. Serum N.P.N was 31 mg% Microscopy of the urinary sediment revealed numerous R.B.C. per high power field. Right ureteric catheterisation revealed an impassable stricture at a distance of about 12 cm above the uretero-vesical junction. Intravenous urography revealed normal excretion of opaque medium on the left side and no excretion on the right. Two years later (1933) lituria recurred and was associated with nausea. Serum N.P.N. was 30 mg% Apart from cystine crystals in the urinary sediment, nothing abnormal was found in the urine. B.P was 130/90 mmHg.

At the age of 40 (1928) the patient was treated in hospital for retrobulbar neuritis. In 1934 she was operated upon for anorectal abscess post-operatively she developed fistula which was resistant to various forms of treatment, including radiotherapy At the age of 50 (1938) she injured her right knee and developed arthritis. B P was then 130 80 mmHg. Serum N P.N was between 31 mg% and 42 mg% Microscopy of the urinary sediment revealed numerous W B C and few cystine crystals per high power field. In 1939 there was no radiographic evidence of renal calculi. In 1943 radiography revealed enlargement of the left kidney the right kidney was not outlined. There was still no radiographic evidence of urinary calculi. B.P was 130 mmHg systolic. Serum N P.N was 31 mg% There was no lbuminuria The urinary sediment did not show any abnormality

Four years later (1947) the patient developed oedema of the legs and albuminuria. B P was 120 80 mmHg. Serum N P N was 56 mg% *Her urine w found to contain albumen but* no sugar Esbach test vealed 6% albumen in the urine Microscopy of the urinary sediment revealed between fifty and one hundred W B C and no or up to fiv R.B C per high power field The patient was given blood transfusions but her B.P continued to fall. She ran downward course and died at the age of 59 (1947), the causes of death being given as amyloidosis, chronic myocarditis, and renal tuberculosis (7). Urine culture for tubercle bacilli by Löwenstein method was negative but guinea-pig inoculation test was positive. Post-mortem examination was not performed.

Urinalysis

1930 Cystine crystals in the sediment (Bjure & Selander).

1931 Cystine crystals in the sediment (Mörner).

1933 The same findings as in 1931 (Mörner).

Stone analysis

1931 *Calculi passed spontaneously that same year* 2 stones weighing 0.45 g and 0.1 g respectively composed of cystine, ash content being 0._% (Mörner).

This case has earlier been reported by Mörner in 1932 (M XXX).

Family XXII

Sixteen individuals and 3 generations were examined.

Results. 2 cases of homozygous cystinuria. N case of semi-cystinuria.

Case A XXII 01 A. C K., a guard, born in 1893 His first attack of renal colic occurred about the age of 30 (19_3). Thereafter he usually developed cystitis whenever he caught a cold. At the age of 53 (1946) he had several further attacks of renal colic, each culminating in the spontaneous passage of calculi. In the following year (1947) he dev loped cystopyelitis associated with frequency of micturition and right renal pain. B P was 160 100 mmHg. That same year a coral stone the size of a pigeon egg was removed from the right renal pelvis by *pyelolithotomy* the operation reveal*ing considerable enlargement of the right kid*ney Post-operatively B.P was 150 105 mmHg his temperature rose to 40 C on two occasions, but after few days treatment with urinary antiseptics it returned to normal. He was given diet to render his urine acid.

Three months after the operation radiography revealed recurrence of calculi in the right kidney which showed impaired function. B.P was 135 85 mmHg. Microscopy of the

urinary sediment revealed a large number of W.B.C., and a few R.B.C. per high power field. Serum N.P.N. was 33 mg%. Thereafter the patient had urinary tract symptoms on and off but these were not associated with pyrexia. When he was 66 years old (1959) radiography revealed extensive necrosis of the right kidney and the presence of calculi therein. B.P. was 145/85 mmHg. Serum creatinine was between 1.4 mg% and 1.6 mg%. Microscopy of the urinary sediment revealed a large number of W.B.C. per high power field. Serum N.P.N. was between 27 mg% and 31 mg%. *Right nephrectomy* was performed. The kidney was found to be smaller than normal and showed mild perinephritis, and moderate degree of hydronephrosis, an additional finding being calculi in the renal pelvis. Thereafter the patient remained well. Urography performed in 1960 revealed no significant abnormality. B.P. was 150/90 mmHg. At the age of 57 (1960) he sustained a fracture of the neck of the right femur.

Urinalysis

1956 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1959: *Calculus removed from the right renal pelvis that same year*: cystine stone (Laboratory at Sahlgren's Hospital, Göteborg).

Case No. XXII 06. G. K., a foreman, born in 1905. He had his first attack of renal colic at the age of 31 (1936). This was followed during the next eight to nine years, by recurrent attacks of right renal colic of increasing severity punctuated by the passage of stones. Accordingly he was admitted to hospital at the age of 40 (1945). B.P. was 175/110 mmHg. Serum N.P.N. was between 29 mg% and 32 mg%. As he continued to have severe attacks of renal colic and developed bacteriuria *right nephrectomy* was carried out a year later (1946). Some of the calculi that had been found in the renal pelvis were preserved but no report on their analysis was available. Microscopically the operation specimen showed signs of hydronephrosis and chronic pyelonephritis. From the age of 45 (1950) there were frequent attacks of left renal colic, culminating in the spontaneous passage of calculi, and the patient was several times admitted to hospital. Creatinine clearance was 126 ml per minute and serum N.P.N. was 29 mg%. At the age of 51 (1956) he developed anuria, caused by a calculus, the size of an orange pip, in the left ureter. After a couple of hours he passed the calculus spontaneously. Thereafter he remained well but at the follow-up examination at the age of 55 (1960) radiography revealed the presence of a coral stone in the left kidney. B.P. was 170/105–95 mmHg. Serum N.P.N. was 36 mg%. The serum creatinine level was between 2.02 mg% and 2.07 mg%, the creatinine clearance being 42 ml per minute. In 1961 *left pyelolithotomy* was performed. Post-operatively serum N.P.N. rose to 75 mg%. Serum creatinine was then between 2.0 mg% and 2.3 mg%. He developed urinary tract infection which was difficult to overcome. Six months later the serum creatinine level was between 1.96 mg% and 2.03 mg%, the creatinine clearance being 46 ml per minute. B.P. was 195/105 mmHg. PAH clearance was between 761 ml and 696 ml per minute. At the age of 57 (1962) he was re-admitted to hospital with cystopyelitis. Serum creatinine was then 1.9 mg% and creatinine clearance 83 ml per minute. Following three days' treatment with sulpha drugs his temperature returned to normal.

In 1959 he developed dyspnoea and myocardial infarction. Treatment with a digitalis preparation and theophylline resulted in an improvement in his condition. During the operation in 1961 the E.C.G. had revealed changes suggestive of myocardial infarction but these regressed quickly. Thereafter he was put on a vasodilator.

Urinalysis

1956 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1955: *Calculi passed spontaneously*: cystine stones (roentgen crystallography, Lagergren).

1961 *Calculus removed from the right renal pelvis in 1946* cystine stone (roentgen crystallography Hambraeus & Lagergren).

1961 *Calculus removed from the left renal pelvis that same year* cystine stone (roentgen crystallography Hambraeus & Lagergren).

1961 *Calculi passed spontaneously* cystine stones (roentgen crystallography Hambraeus & Lagergren).

D Y ON RELATIVES OF THE ABOVE CYSTINURICS

The paternal grandfather and grandmother of the cystinurics were cousins.

Family XXIII

Twelve individuals and 3 generations were examined.

Results: 3 cases of homozygous cystinuria. *No case of semi-cystinuria.*

Case No. XXIII 01 02 G.I.W a housewife born in 1908. The patient was married and had one child (1 baby was stillborn). She had several episodes of cystitis, there being no history of other urinary tract symptoms

At the follow-up examination in June 1960 she was in good condition and had no symptoms of physical disease. B.P was 140/90 mmHg.

Urinalysis

1956 and 1960 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Case No XXIII 01 04 K.E.O a housewife, born in 191.. The patient was married and had one child At the age of 35 (1947) urinary tract symptom appeared in the form f several episodes of pyelitis. That same year she was admitted to hospital with albuminuria and pyelonephritis. Radiography revealed bilateral renal calculi. B.P was 140/80 mmHg. Her urine was positive for albumen but negative for sugar Microscopy of the urinary sediment revealed a large number of R.B.C. and several W.B.C. per high power field. There was no bacteriuria. At the age of 36 (1948) she underwent *left pyelolithotomy* 1 large and 8 small calculi being removed. Post-operatively she had an attack of right renal colic, culminating in the spontaneous passage of calculi. Four weeks later *right pyelolithotomy* was carried out, the operation revealing dilatation of the right renal pelvis which contained one large and several small calculi. Less than a month after the operation a further attack of renal colic recurred associated with pyrexia of 40 C. She was re-admitted to hospital and treated with antibiotics. Microscopy of the urinary sediment revealed several W.B.C. and several bacteria (rods) per high power field. Thereafter she was followed up as an out-patient. Urinary tract infection persisted and she had mild attacks of renal colic off and on. Radiography carried out three years after the second operation (1951) revealed that calculi had reformed in the left kidney At the age of 40 (1952) there were episodes of pyelitis associated with high fever A calculus was found to be blocking the proximal left ureter Her urine wa found to contain albumen but no sugar Microscopy of the urinary sediment revealed large number of R.B.C. and a few bacteria (rod) per high power field. A second *left pyelolithotomy* was performed, the operation revealing fibrous perirenal adhesions and fibrosi of the renal pelvis. Several small calculi were removed Tw months later pyelitis recurred and the patient passed calculi spontaneously She was successfully treated with antibiotics. Radiography revealed the presence of calculi in the left kidney Serum N P.N was 29 mg% Serum calcium was 9.9 mg% serum phosphorus 2.3 mg% and the creatinine clearance 104 ml per minute

In 1953 radiography showed that calculi had reformed bilaterally Serum calcium was 10 0 mg% serum phosphorus 2 6 mg% and creatinine clearance 126 ml per minute. B P was 190 110 mmHg. As the calculus in the right kidney had increased in size *right partial nephrectomy* and *pyelolithotomy* were performed, the patient being then 42 years old (1954). Serum N P.N was between 23 mg% and 30 mg% Post-operatively she wa given

medicines to render her urine acid because the removed calculus was thought to be an infection stone, but when analysis revealed it to be a cystine stone the acidifying drugs were immediately withdrawn. Thereafter the patient was followed up for two years and was found to be well apart from hypertensive symptoms, B.P being 180–200/110 mmHg (1956). Serum N.P.N was 33 mg% However further episodes of pyelitis and attacks of renal colic associated with haematuria and ending in the spontaneous passage of gravel and stones occurred. A third *pyelolithotomy* was performed on the left side the operation revealing marked dilatation of the renal pelvis. She was prescribed a high fluid intake *ad modum* Dent & Senior (1955) and remained relatively well while on this regime. At the age of 46 (1958) radiography revealed coral stone in the right kidney which w removed by *pyelolithotomy* B.P was 180/110 mmHg. Both kidneys showed satisfactory function. Serum N.P.N was between 35 mg% and 40 mg% Thereafter she was symptom-free but radiography performed after this last operation revealed a recurrent calculus in the right kidney which two years later (1960) was removed by *pyelolithotomy* B.P was 230/130 mmHg. Serum N.P.N was between 33 mg% and 40 mg% Her urine was positive for albumen. Microscopy of the urinary sediment revealed few R.B.C. and numerous W.B.C. per high power field. Thereafter the patient remained subjectively relatively well and was instructed to continue her high fluid intake.

At the follow-up examination in 1960 she was in relatively good condition. She was obese and had t that time no urinary tract symptoms. B.P was 210 130 mmHg.

Urinalysis

1954 Abnormal amounts of cystine cysteine, lysine, arginine and ornithine (Pernow).

1956 and 1960. Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

1952: *Calculus removed from the left renal pelvis that same year*: cystine stone (roentgen crystallography Lagergren).

1954 *Calculus removed from the right renal pelvis that same year*: cystine stone (roentgen crystallography Lagergren).

1956. *Calculus removed from the left renal pelvis that same year*: composed of cystine, large amounts of struvite and apatite being present in addition (roentgen crystallography Lagergren).

1958 *Calculus removed from the right renal pelvis that same year*: infection stone composed of struvite and apatite cystine being absent (roentgen crystallography Lagergren).

1958 *Calculus removed from the right renal pelvis that same year*: infection stone composed of struvite and apatite, containing small amount of cystine in addition (roentgen crystallography Lagergren).

1960: *Calculus removed from the right renal pelvis that same year*: infection stone composed of struvite and apatite, showing a small amount of cystine (roentgen crystallography Hambraeus & Lagergren).

Case No XXIII 01·07 R.O.M a house wife, born in 1924 The patient was married and had one child, she enjoyed good health. There was no history of urinary tract symptoms.

Urinalysis

1958 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Family XXIV

Twenty-two individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. N case of semi-cystinuria.

Case No. XXIV 02 01 E.Ö a house-wife, born in 1914 The patient was married and had two children. At the age of 29 (1943) she was admitted to hospital with thyrotoxicosis. Eleven years later (1954) she underwent partial thyroidectomy Her condition did not improve and she was several times treated in hospital for paroxysmal tachycardia, neu-

rasthenia, and anxiety At the age of 40 (1954) she had haematuria and two attacks of right renal colic. That same year a calculus was removed from the right renal pelvis by *pyelolithotomy* the operation revealing the renal pelvis to be slightly dilated. Post-operatively attacks of renal colic occurred on either side, culminating in the spontaneous passage of calculi. Radiography revealed a calculus, the size of pinhead, in the right kidney A year later (1955) there were further attacks of renal colic associated with haematuria. Urography revealed a calculus, the size of a hazelnut, in the right kidney Serum calcium was between 9.. mg% and 13 7 mg% serum phosphorus between 2.5 mg% and 4.4 mg% and the 24-hour urinary output of calcium 148.8 mg. B.P was 160/90 mmHg. Serum N.P.N was 27 mg% Microscopy of the urinary sediment revealed numerous R.B.C. and about ten W.B.C. per high power field. The calcium tolerance test revealed normal parathyroid function. As the patient continued to have attacks of renal colic the calculus in the right kidney was removed by *pyelolithotomy* the operation revealing marked enlargement of the renal pelvis and oedema of the mucous membrane. Immediately after the operation the patient passed stone spontaneously Thereafter she maintained high urinary output and has remained well.

At the follow-up xamination in June 1960 she was in good condition and had no urinary tract symptoms. B.P was 160 100 mmHg.

Urinalysis

1956 and 1960· Abnormal amounts of cystine, lysine, arginine, and ornithine (Bostrom & Hambraeus).

Stone analyses

1954 *Calculus removed from the right renal pel is that same year* yellowish-brown stone, 28 mm 20 mm 15 mm in size, with a finely nodular surface, its shape being irregularly triangular It was not analysed.

1955 *Calculus removed from the right renal pelvis that same ear* a small crystalliform cystine stone of light brow colour .3 mm 14 mm 10 mm in size (Mollerberg).

1961 *Calculus passed spontaneously* cystine stone (roentgen crystallography Hambraeus & Lagergren).

Family XXV

Eight individuals and 3 generations were examined.

Results 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XXV 01 03 M.C.L.T an engineer born in 1929 His first attack of renal colic occurred at the age of 21 (1950). A year later a calculus, the size of hazel-nut, was removed from the left kidney by combined *pyelo- and ureterolithotomy* A few weeks later an attack of right renal colic occurred. Radiography revealed that there was no excretion of opaque medium from the right kidney Three years later (1954) he experienced occasionally right renal pain associated with haematuria and the spontaneous passage of calculi. Radiography revealed bilateral coral stone. A year later (1955) *right pyelolithotomy* was performed. Post-operatively further attacks f right renal colic occurred and second *right pyelolithotomy* was performed. B.P w 140 85 mmHg. Serum N.P.N was between ?7 mg% and 3 mg% At the age of 30 (1959) he developed pyelitis. H reported that he felt better when on vegetable diet.

At the follow-up examination in June 1960 the patient was in good condition and had no urinary tract symptoms. B P was 120/70 mmHg.

Urinalysis

1956 and 1960: Abnormal amounts of cystine, lysine, arginine and ornithine (Bostrom & Hambraeus).

Stone analysis

1955 *Calculus removed from the right renal pelvis by the first peration.* cystine stone (Swedin).

1955 *Calculus removed from the right renal pelvis by the second operation.* cystine stone (roentgen crystallography Lagergren).

Family XXVI

Twenty-six individuals and 3 generations were examined

Results: 3 cases of homozygous cystinuria. 1 case of semi-cystinuria.

Case No. XXVI 03 05 S. H. L., a farmer born in 1917 Apart from scarlet fever com plicated by albuminuria at the age of 24 (1941) he had been healthy When he was 34 years old (1951) he began to have urinary tract symptoms in the form of ttacks of left renal colic and frequency of micturition. The fol lowing year (1952) he was admitted to hospital with acute renal colic and oliguria. Urography revealed the presence of calculi in both renal pelves and a calculus in the left ureter which was causing left hydronephrosis. There also was radiographic evidence of a calculus in the bladder The radiographic studies revealed poor concentration and delayed excretion of opaque medium bilaterally B.P was 145/90 mmHg. Serum N P.N was between 44 mg% and 38 mg% There was albuminuria. Microscopy of the urinary sediment revealed a few R.B.C. and numerous W.B.C. per high power field. Five calculi, each about the size of a walnut, were removed from the left ureter by *ureterolithotomy* The patient was treated with antibiotics and was discharged home symptom free about four weeks after the operation. About two weeks after discharge home he was re-admitted with pyrexia and diffuse abdominal pain. Radiography revealed calculi in both renal pel ves and a calculus in the bladder There was radiographic evidence of bilateral hydronephrosis, particularly on the right side. B.P was 120/70 mmHg. Serum N.P.N was between 43 mg% and 30 mg% serum calcium 10 1 mg% and serum phosphorus 3 4 mg% There was albuminuria. Microscopy of the urinary sediment revealed few R.B C., numerous W.B.C., and bacteria (coli) per high power field. A coral stone was removed from the right kidney by *pyelol thotomy* and the vesical calculus by *suprapubic cystolithotomy* Post operatively there was again radiographic evidence of bilateral hydronephrous, particularly on the right *side* The patient was given antibiotics and medicines to render his urine acid.

Six months after his discharge home he was re-admitted (1953) with an attack of left renal colic and pyrexia. He had had several similar though less severe attacks during the previous few months. Radiography revealed that a calculus in the left kidney had moved down into the distal part of the left ureter B.P was 120/80 mmHg. Serum N.P.N was between 22 mg% and 36 mg% serum calcium 10 8 mg% and serum phosphorus 2.2 mg% There was albuminuria. Microscopy of the urinary sediment revealed a few R.B.C., numerous W.B.C., and bacteria (rods and cocci) per high power field. *Left ureterolithotomy* was performed, the operation revealing that the ureter was surrounded by firm connective tissue. The calculus could not be removed. The ureter was divided and re-implanted into the bladder The post-operative course was uneventful. He was given antibiotics and drugs to acidify the urine. He was discharged home on course of a sulpha preparation. That same year the patient was admitted further three times to the same hospital with cystopyelitis. B.P was then about 170/80 mmHg. Serum N P.N was between 30 mg% and 43 mg% There was albuminuria. He was treated with antibiotics. At the age f 37 (1954) he was again admitted to hospital with cystopyelitis and right-sided abdominal pain. Serum N.P.N was between 48 mg% and 58 mg% The f llowing year (1955) he was admitted to hospital with right-sided abdominal pain associated with pyrexia. Serum N P.N was then 88 mg% B.P was 160/90 mmHg. The patient s condition deteriorated, serum N.P.N rose to 174 mg% and he died at the age of 38 the cause of death being given as uraemia. Post-mortem examination was not performed.

Urinalysis

Not performed.

Stone analysis

N stones available.

The history of this patient was considered to be sufficiently characteristic to permit the diagnosis of cystinuria to be made (Hambraeus, 1960).

Case No. XXVI 03 07 K.O.W a dairy man, born in 1919 At the age of 22 (1941)

he had scarlet fever. There were no complications. At the age of 37 (1956) he developed symptoms suggestive of renal colic. Urography did not reveal any abnormality. The urine was found to contain a trace of albumen but no sugar. Microscopy of the urinary sediment revealed some R.B.C. and a few W.B.C. per high power field. There was no bacteriuria. Serum N.P.N. was 30 mg%. At the age of 43 (1962) he had an attack of left renal colic. There was also history of occasional gastritis.

At the follow-up examination in May 1960 he was in good condition and did not show any signs of physical disease. B.P. was 130/90 mmHg.

Urinalysis

1956: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

1956: A large number of cystine crystals in the urine (Laboratory at the Hospital in Falköping).

1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

Not performed.

Case No. XXVI 03 09. I. A. M. N., a housewife, born in 1926. The patient was married and had one child. At the age of 14 (1941) she had scarlet fever. There were no complications. Apart from a few episodes of cystitis she had remained well.

At the follow-up examination in May 1960 the patient's general condition was found to be good and she had no urinary tract symptoms. B.P. was 130/80 mmHg.

Urinalysis

1957: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Case No. XXVI 03 11. A. H. I. L., shop-assistant (male), born in 1928. At the age of 13 (1941) he had scarlet fever complicated by albuminuria. Since the age of 15 he had had backache. At the age of 22 (1950) he was admitted to hospital on account of recurrent attacks of right renal colic and occasional haematuria. Radiography revealed calculi in the renal pelvis on either side, both renal pelves being shown to be dilated. There was poor excretion of opaque medium on the left side, which was probably due to a calculus obstructing the ureter. Serum N.P.N. was within normal limits. B.P. was 155/100 mmHg. A large calculus and about 50 smaller calculi, varying in size from those of peas to those of hazel-nuts, were removed from the left renal pelvis by pyelolithotomy. Postoperatively he passed several small calculi spontaneously. Radiography revealed normal function of the right kidney only. In the following three months three attacks of right renal colic occurred, each ending in the spontaneous passage of calculi, there being no symptoms of urinary tract infection. At the age of 23 (1951) he was again admitted to hospital. Urography revealed poor excretion of opaque medium on the right side; the left kidney showed normal function. There was albuminuria. B.P. was 190/110 mmHg. Microscopy of the urinary sediment revealed a large number of W.B.C. and bacteria (cocci) per high power field. There was radiographic evidence of multiple calculi in the right renal pelvis; one calculus was seen partly to project into the ureter. The left renal pelvis contained about 70 calculi the size of cherries. A calculus, the size of a plum, and a large number of smaller calculi were removed from the right renal pelvis by pyelolithotomy. As there was post-operatively still radiographic evidence of the presence of calculi in the right kidney, pyelolithotomy was again performed after an interval of three months. Urography carried out after this last operation showed that there was no excretion of opaque medium on the right side. There was albuminuria. The urinary sediment did not show any abnormality. The patient remained well until 1954 when he developed epididymitis on the left side. Microscopy of the urinary sediment revealed one or two R.B.C., numerous W.B.C., and bacteria (rods) per high power field. Serum N.P.N. was 33 mg%. Radiography revealed multiple calculi in the left

kidney but none in the right. Urography revealed delayed excretion of opaque medium on the right side and bilateral hydronephrosis, due to obstruction at the pelvi-ureteric junction. The calculi were removed from the left kidney by *pyelolithotomy* that same year (1954).

Two years later (1956) he was again admitted to hospital with an attack of left renal colic. There was albuminuria. Microscopy of the urinary sediment revealed one or two W.B.C. and few bacteria (rods) per high power field. During the first week of his stay in hospital serum N.P.N. was between 45 mg% and 64 mg% but it later fell to 33 mg%. Serum calcium was between 9.5 mg and 11 mg%. There was radiographic evidence of the presence of calculi in the left renal pelvis. As the patient refused to be operated upon he was discharged home but was re-admitted on account of recurrence of severe attacks of renal colic two weeks later. Radiographic studies revealed 4 calculi in the left kidney and delayed excretion of opaque medium and hydronephrosis on the right side. A water dilution test revealed impaired renal function. B.P. was 170/80 mmHg. His urine was found to contain albumen but no sugar. Cystine crystals were present in the urinary sediment. Serum N.P.N. was 34 mg%. The left renal calculi were removed by *pyelolithotomy*. Post-operatively there was a short-lived elevation of serum N.P.N. to 57 mg%. As the extracted calculi were found to be cystine stones the patient was put on high fluid intake and sodium citrate. At the follow-up examination four months later the serum calcium and phosphorus levels were found to be normal. Urography did not reveal any abnormality and there was no evidence of recurrence of renal calculi. B.P. was 140/90 mmHg. His urine was found to contain albumen but no sugar. Microscopy of the urinary sediment did not reveal any abnormality. Serum N.P.N. was between 35 mg% and 40 mg%.

Thereafter he had no further attacks of renal colic, but albuminuria persisted and he was therefore regularly followed up. He began to drink heavily and contemplated suicide. At the age of 31 (1959) he was admitted to hospital on account of undue fatigue, deterioration of vision, generalized oedema, pruritus, and severe albuminuria. On admission his B.P. was 00/100 mmHg; ophthalmoscopy revealed a few exudates but the discs and arteries were normal. Haemoglobin was 46%. Serum N.P.N. was 106 mg%, serum creatinine 14.4 mg%, and serum albumen 5.6 mg%. He suffered from excruciating pain in the region of the pericardium and dyspnoea. He was given several blood transfusions, but serum N.P.N. rose to 148 mg%. He went downhill and died that same year at the age of 31, the cause of death being given as uraemia. Post-mortem examination revealed severe pericarditis, bilateral perirenal adhesions on either side, extensive fibrotic degeneration of the left kidney and cystic dilatation of the right kidney which contained about 00 ml of purulent light green fluid, only a thin rim of renal parenchyma remaining on either side.

Urinalysis

1956. Abnormal amounts of cystine (Hellsing-Lenskjöld).

1957. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1957. *Calculus removed from the left kidney that same year*: cystine stone (roentgen crystallography, Lagergren).

This case has earlier been reported by Hambraeus & Boström (1960).

Case No. XXI 1 03 12. M. G. K. A., a house-wife born in 1930. The patient was married but childless. She had had a single pregnancy which was complicated by toxaemia of pregnancy and intra-uterine foetal death. At the age of 11 (1941) she had scarlet fever. There were no complications. At the age of 14 she had her first attack of renal colic which involved the right side and ended in the spontaneous passage of a calculus. B.P. was then 135/80 mmHg and serum N.P.N. 25 mg%. Her urine was positive for albumen. Microscopy of the urinary sediment revealed one or two R.B.C. and a few W.B.C. per high power field, bacteria being absent.

Urinalysis

1957 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

Not performed.

Family XXVII

Forty seven individuals and 4 generations were examined.

Results: 1 case of homozygous cystinuria. 1 case of semi-cystinuria.

Case N XXVII 01 04 01 B.S. F watchmaker born in 1938 The patient had weighed 3,005 g at birth and 2,600 at the age of four months. As an infant he had pertussis and as a child attacks of asthma. At 6 months he was admitted to hospital with dyspepsia. At the age of 9 months he developed pyelocystitis which was treated with urinary antiseptics. Radiography revealed the presence of 2 urinary calculi, one in the right kidney and the other in the left ureter the former being removed by *pyelolithotomy* and the latter after an interval of four weeks, by *ureterolithotomy* Post-operatively he developed pyelitis associated with pyrexia but radiographic studies did not reveal any abnormality The following year (1940) he was re-admitted to hospital on account of exacerbation of his urinary tract symptoms As there was radiographic evidence of marked hydro-ureter due to an obstructing calculus on the right side *ureterolithotomy* was performed. From 1940 to 1949 he was twelve times admitted to hospital on account of recurrent attacks of renal colic and recurrent cystopyelitis. At the age of 16 (1954) he had again an attack of renal colic which involved the left side. Radiography revealed the presence of calculi, one in the right renal pelvis and the other in the left ureter which was causing almost complete obstruction. Serum N.P.N was between 29 mg% and 35 mg%. Left *ureterolithotomy* was again performed. Thereafter he remained well for five years. In 1959 it was noted that the output of urine in the morning was very small. An increase in the fluid intake during the night was ineffective. Shortly after he had been put on high fluid intake attacks of bilateral renal colic occurred being occasionally associated with haematuria. Serum N.P.N was 36 mg% Creatinine clearance was 84 ml per minute. B.P was 170/80–75 mmHg. As there was radiographic evidence of a calculus in the left ureter which was causing obstruction, *ureterolithotomy* was again performed.

In the summer of the year 1960 he developed mild prostatitis. At a follow-up examination in September that same year he was found to be in good condition there was no tenderness in the region of the kidneys and he had no urinary tract symptoms. B.P was 115/70 mmHg. On culture of the urine moderate growth of enterococci was obtained. From 1961 to 1962 he had several attacks of renal colic ending in the spontaneous passage of calculi. On one occasion in 1961 a calculus got held up in the urethra. As it caused anuria it was extracted. He was again admitted to hospital with urinary tract infection that same year (1961) and was put on course of sulpha drug. B.P was 120/80 mmHg, serum creatinine 1.27 mg% and creatinine clearance 64 ml per minute. Urography in 1961 and 1962 revealed evidence of the presence of a calculus in the right renal pelvis.

Urinalysis

1940 Cystine crystals in the sediment (Laboratory at Kronprinsessan Lovisa Barnsjukhus, Stockholm).

1956 and 1960 Abnormal amounts of cystine lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1939 *Calculus removed from the left ureter 1938* the size of a pea showing a smooth surface and composed of calcium carbonate and calcium oxalate (Agner).

1939 *Calculus removed from the right renal pelvis in 1938.* brittle the size of hazel-nut kernel, and composed of calcium carbonate and calcium oxalate (Agner).

1954 *Calculus removed from the left ureter that same year* composed predominantly of cystine, small amounts of apatite being present

in addition (roentgen crystallography Lagergren).

1959 *Calculus removed from the left ureter that same year* cystine stone (roentgen crystallography Lagergren).

1961 *Calculus removed from the urethra in 1960* yellowish-brown stone, weighing 0.61 g, measuring 13 mm 9.3 mm 6.3 mm in size, its surface being finely nodolar and glossy Not analysed.

Family XXVIII

Eleven individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria (screening-case).

No case of semi-cystinuria.

Case No XXVIII. 01 02.01 P L.T a school-girl, born in 1950. She had no urinary tract symptoms.

Urinalysis

1957 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambræus).

Stone analysis

No stones available.

Family XXIX

Four individuals and 3 generations were examined.

Results: 2 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No XXIX 01 04 F K., male patient, born in 1894 who emigrated to U.S.A. At the ages of 34 (1928) 49 (1943), and 66 (1960) he had passed urinary calculi spontaneously At the age of 67 (1961) he developed left ureteric obstruction which was caused by a calculus and was associated with haematuria. The ureteric calculu was removed by *ureterolithotomy* Post-operatively he passed a stone spontaneously Apart from arthritic symptoms he remained well thereafter

Urinalysis

Not performed.

Stone analysis

1961 *Calculus emoved from the left ur ter that same year* cystine stone (analysed in U.S.A.).

1961 *Calculus passed spontaneously that same year* cystine stone (analysed in U.S.A.).

Case No. XXIX. 01 05 O W K., a businessman, born in 1897 He had his first attack of renal colic at the age of 39 (1936); it involved the right side. Serum N.P.N was 30 mg% There was no albuminuria. Microscopy of the urinary sediment revealed one or two R.B.C. and ten W.B.C. per high power field. Radiography revealed the presence of calculi in the right renal pelvis, 3 being removed from the latter by *pyelolithotomy* Thereafter he remained well for several years. Right renal ache eventually recurred and at the age of 46 (1943) radiography revealed the re currence of a calculus in the right renal pelvis. There was no albuminuria. Microscopy of the urinary sediment revealed one or two R.B.C. and numerous W.B.C. per high power field. Serum N.P.N was 36 mg% B.P was 140/100 mmHg. As the calculus did not cause any noteworthy discomfort to the patient he was not operated upon until six years later (1949) when it was removed by *pyelolithotomy* Thereafter attacks of right renal colic recurred and occa sionally he experienced bilateral renal ache. At the age of 54 (1951) he developed symptoms suggestive of the presence f gallstones and was admitted to hospital for investigation. Serum N.P.N was 34 mg% Thereafter he remained well until the age of 58 (1955) when there was microscopic haematuria, renal ache being absent. Serum N.P.N was 33 mg% *Right nephrectomy* was performed, the operation revealing a small kidney adherent to surrounding tissue with a dilated renal pelvis containing multiple calculi. The post-operative course was uneventful. Serum N.P.N was 29 mg% Thereafter he was symptom-free.

At the follow-up examination in M y 1960 the patient was in good condition and did not have any urinary tract symptoms. B.P was 150 85 mmHg. Radiography showed no evidence of calculi in the left kidney

Urinalysis

1957 and 1960: Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

1949: *Calculus removed from the right renal pelvis that same year*: cystine stone (G. Hammarsten).

1955 *Calculus removed from the right renal pelvis that same year*: cystine stone (G. Hammarsten).

1961 *Calculus removed from the right renal pelvis in 1936*: cystine stone (roentgen crystallography Hambraeus & Lagergren).

1961 *Calculus removed from the right renal pelvis in 1949*: *cystine stone (roentgen crystallography* Hambraeus & Lagergren).

1961 *Calculus removed from the right renal pelvis on nephrectomy in 1955*: composed predominantly of cystine, small amounts of apatite being present in addition (roentgen crystallography Hambraeus & Lagergren).

Family XXX

Seventy individuals and 4 generations were examined.

Results: 1 case of homozygous cystinuria. 2 cases of semi-cystinuria.

Case N XXX 01 05 E. A. O. housewife, born in 1924. The patient was married and had two children. At the age of 11 (1935) she had poliomyelitis resulting in right hemiplegia and had been confined to her bed for six months. At the age of 16 (1940) she developed gnawing pain in the region of the right kidney and occasionally passed calculi spontaneously. During her pregnancy in 1950 her urinary tract symptoms exacerbated, frequency of micturition being an additional symptom. Eight days after parturition the urinary tract symptoms recurred being associated with pyrexia to 40.4 C. Radiography revealed the presence of a large calculus in the right ureter which was causing ureteric obstruction, and was removed by *ureterolithotomy*. The post-operative course was uneventful. Serum N.P.N. was 30 mg%. From 1954 to 1960 she was regularly followed up by radiography which invariably revealed the presence of bilateral calculi. In 1955 she underwent therapeutic abortion, the indication being her urolithiasis.

Apart from an attack of renal colic in 1957 which ended in the spontaneous passage of a calculus, the patient remained well until 1960 when she developed dysuria and right renal ache recurred. Three months later these symptoms were associated with pyrexia (38.6 C) and she was therefore admitted to hospital. B.P. was 115/75 mmHg. There was albuminuria. Microscopy of the urinary sediment revealed a large number of W.B.C. and one or two R.B.C. per high power field. *Radiography revealed the presence of 4 calculi in* the right upper urinary tract, 1 in the renal pelvis, 1 at the level of the pelvi-ureteric junction and 2 in the distal part of the ureter. Combined *pyelolithotomy* and *ureterolithotomy* were carried out. The urinary infection was treated with antibiotics. The post-operative course was uneventful. Thereafter she remained well until 1961 when she developed left-sided renal ache. Radiography revealed the presence of a calculus at the level of *the left pelvi-ureteric junction. B.P.* was 150/80 mm Hg. The calculus was removed by *pyelolithotomy*. The post-operative course was uneventful.

Thereafter she was symptom-free until 1962 when a further attack of left renal colic recurred. B.P. was 135/85 mmHg. Radiographic studies did not reveal any abnormality of the right kidney but there was evidence of the presence of calculi in the left ureter, delayed excretion of opaque medium on the left side, and above the site of the calculus dilatation of both the renal pelvis and ureter. *Ureterolithotomy* was carried out removing 1 large and small calculi from the left ureter. The post-operative course was uneventful. At the follow-up examination performed six months later (196.) she was found to have symptoms of cystitis. B.P. was 140/80 mmHg. Radiography revealed the recurrence of a calculus in the left ureter, from which it was removed by *ureterolithotomy*. The post-operative course was uneventful.

Urinalysis

1937 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1948. *Calculus passed spontaneously*: cystine stone (Silwer).

1954. *Calculi passed spontaneously*: cystine stones (G. Hammarsten).

1954. *Calculus removed from the right ureter in 1950*: cystine stone (Hanson).

1960: *Calculus removed from the right renal pelvis that same year*: cystine stones (Laboratory at the Hospital in Lund).

1961. *Calculus removed from the left renal pelvis that same year*: cystine stone (Laboratory at the Hospital in Lund).

1961. *Calculi passed spontaneously*: cystine stones (roentgen crystallography: Hambraeus & Lagergren).

1962. *Calculus removed from the left ureter that same year*: cystine stone (Laboratory at the Hospital in Lund).

Family XXXI

Fifteen individuals and 3 generations were examined.

Results. 3 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No. XXXI 01. E. C. E., a house-wife born in 1891. The patient was married and had two children. At the age of 33 (1924) she had her first attack of renal colic. This was followed by about 12 further attacks, each ending in the passage of gravel. She was neither operated upon nor X-rayed. In the last few years she had had cardiac and rheumatic symptoms.

Urinalysis

1957. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

Not performed.

Case No. XXXI 0[illegible]. K. A. L., a builder labourer born in 190[illegible]. Since childhood he had been forming either vesical or renal calculi. At the ages of 4 (1906) and 25 (1927) calculi were removed from the bladder by *suprapubic cystolithotomy*. At the age of 26 (1929) a vesical calculus got held up in the urethra and had to be extracted. Thereafter he passed gravel off and on. At the age of 48 (1950) he had an attack of right renal colic associated with macroscopic haematuria. Serum N.P.N. was between 33 mg% and 40 mg%. Radiography revealed a calculus in the right renal pelvis from which it was removed by *pyelolithotomy*. Post-operatively he developed a urinary fistula in the wound which required treatment in hospital. The following year (1951) attacks of right renal colic recurred. Radiography revealed the presence of multiple calculi in the right renal pelvis. He passed several calculi spontaneously but on two occasions a calculus got held up in the urethra and had to be extracted. As attacks of right renal colic continued to occur *pyelolithotomy* (1952) was again carried out. His urine was found to contain albumen but no sugar. Microscopy of the urinary sediment revealed between five and eight R.B.C. and between three and five W.B.C. per high power field. Serum N.P.N. was between 40 mg% and 50 mg.

Thereafter he developed hypertension associated with nausea and vomiting, and in 1956 and 1957 he was on several occasions admitted to hospital for treatment of these symptoms. B.P. was then 230/130 mmHg and serum N.P.N. 73 mg%. His urine was found to contain albumen but no sugar.

In the beginning of 1957 serum N.P.N. was 108 mg% rising to [illegible]43 mg% shortly before his death. B.P. was then 210/140 mmHg. Microscopy of the urinary sediment revealed from two to three R.B.C. and three to four W.B.C. and bacteria (rods) per high power field.

His condition deteriorated and he died at the age of 55 in 1957, the causes of death being given as uraemia and chronic nephritis.

Urinalysis

195[illegible]. Cystine crystals in the urine.

1957. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1952: *Calculus removed from the bladder in 1927* composed predominantly of cystine, traces of calcium magnesium phosphate being present in addition (Laboratory at Karolinska sjukhuset, Stockholm).

1952. *Calculus passed spontaneously* cystine stone (Laboratory at Karolinska sjukhuset, Stockholm).

1952. *Calculus removed from the right renal pelvis that same year* cystine stone (Laboratory at Karolinska sjukhuset, Stockholm).

Case No XXXI 03 01 J A.L., a male patient, born in 1942. Since the age of 7 he had experienced diffuse dull pain in the region of both kidneys which occasionally increased in severity At the age of 13 (1955) he developed haematuria associated with frequency of mic turition and pain in both inguinal regions. B.P was 120 65 mmHg. There was no radiographic evidence of renal calculi. After discharge from hospital he consulted homeopathist who prescribed a diuretic. Thereafter he passed numerous small calculi spontaneously Two years later (1957) urography revealed coral stone the left kidney

Since the age of 7 he had been obese. At the ages of 11 (1953) and 15 (1957) he was admitted to hospital with suspected dystrophia adiposo genitalis, but the cause of his over weight could not be demonstrated. In 1957 creatinine clearance was 140 ml per minute and B.P was 160 80 mmHg.

Urinalysis

1957 Numerous cystine crystals in the sediment (Laboratory at Karolinska sjukhuset, Stockholm).

1957 Abnormal amounts of cystine, lysine, arginine, and ornithine (Bostrom & Hambraeus)

Stone analysis

Not performed.

Family XXXII

Nine individuals and 3 generations were examined.

Results cases of homozygous cystinuria. No case of semi-cystinuria.

Case No. XXXII. 01 03 H.O L.L., a foreman, born in 1923 At the age of 27 (1950) he had poliomyelitis resulting in complete paralysis which subsided apart from the paralysis of the left arm. Shortly after he became ill with poliomyelitis he had an attack of left renal colic ending in the spontaneous passage of gravel. Serum N.P.N was then 54 mg% Radiography did not reveal any urinary tract bnormality Five years later (1956) he had again an attack of left renal colic. Radiographic studies then revealed calculus in the left ureter which was causing ureteric obstruction. Serum N.P.N was 41 mg% His urine was negativ for both albumen and sugar Microscopy of the urinary sediment revealed from eight to ten R.B.C., and no or up to four W.B.C. and numerous cystine crystals per high power field. The ureteric calculus was removed by left *ureterolithotomy* Thereafter he experienced occasionally bilateral renal ache and passed gravel spontaneously

At the follow-up examination in May 1960 he was in good condition and had no urinary tract symptoms. Paralysis of the left arm per sisted with associated muscular atrophy B P was 130 95 mm Hg.

Urinalysis

1956 Cystine crystals in the sediment (Laboratory t the Hospital in Linköping).

1957 and 1960. Abnormal amounts f cystine lysine, arginine and ornithine (Bostrom & Hambraeus).

Stone analysis

1950- *Gravel passed spontaneously that same ear* composed of calcium oxalate (Laboratory at the Hospital in Umeå).

Case N XXXII. 01 04 R.O L., factory hand, born in 19 4 At the ge of 21 (1945) he had dysuria nd an attack of renal colic ending in the spontaneous passage of calculus. Thereafter he had no urinary tract symptoms until 1947 when an ttack of right renal colic occurred which culminated in the spontaneous passage of several small calculi. At the age of .6 (1950) he had an ttack of left renal colic. Radiography revealed cal

culus, the size of an almond, in the left ureter which was causing ureteric obstruction and was removed by *ureterolithotomy*. The operation revealed marked hydronephrosis of the left renal pelvis. Serum N.P.N. was 34 mg%. Six months later (1950) he again had an attack of right renal colic associated with haematuria. Radiography disclosed a calculus, the size of a chestnut, in the right renal pelvis from which it was removed by *pyelolithotomy*, a large quantity of gravel being washed out from the renal pelvis in addition. The operation revealed dilatation of the renal pelvis. As there also was urinary infection he was put on antibiotics and medicines to render his urine acidic. Attacks of right renal colic with the spontaneous passage of calculi continued to occur. Serum calcium was 12.5 mg% and serum N.P.N. between 50 mg% and 3 mg%. His urine was found to contain albumen but no sugar. Microscopy of the urinary sediment revealed numerous R.B.C., W.B.C., and cystine crystals per high power field. Serum calcium was between 9.4 mg% and 9.9 mg%, serum phosphorus 3.7 mg% and creatinine clearance 148 ml per minute. As hyperparathyreoidism was suspected partial parathyroidectomy (1951) was carried out. In view of the recurrence of attacks of right renal colic *pyelolithotomy* was again performed that same year (1951). Serum N.P.N. was 35 mg%. Thereafter the patient was symptom-free for four months when an attack of severe renal colic associated with haematuria occurred. His urine was found to contain a trace of albumen but no sugar. Serum N.P.N. was 39 mg and B.P. 155/100 mmHg. As there was radiographic evidence of ureteric obstruction on the right side he was again operated on that same year (1951), 1 calculus being removed from the right ureter and 3 calculi from the renal pelvis by *ureterolithotomy* and *pyelolithotomy* respectively. Post-operatively he continued to have attacks of renal colic culminating in the spontaneous passage of calculi. He regularly attended hospital for follow-up examinations. B.P. was 130–145/70 mmHg, serum N.P.N. was 37 mg and creatinine clearance 123 ml per minute (195). In 1954 there was again an attack of renal colic ending in the spontaneous passage of calculi. Radiography revealed bilateral renal calculi but there was no evidence either of impaired renal function or of ureteric obstruction. B.P. was 130/75 mmHg and serum N.P.N. 29 mg%. Microscopy of the urinary sediment revealed one or two R.B.C. and W.B.C. per high power field, cystine crystals being absent. The following year (1955) there were several episodes of haematuria, and he was therefore admitted to hospital for further investigation. The radiographic appearances were the same as in the preceding year (1954). B.P. was 135/75 mmHg and serum N.P.N. 35 mg%.

In 1957 he had again an attack of renal colic associated with macroscopic haematuria. B.P. was 155/100 mmHg. Microscopy of the urinary sediment revealed a large number of R.B.C. per high power field. Radiography revealed calculi in both renal pelves, and a calculus in the left ureter from which it was removed by *ureterolithotomy*. The postoperative course was uneventful.

Since the age of 28 (1952) duodenal ulceration had been co-existing, being treated medically three years later (1955).

At the follow-up examination in June 1960 he was in comparatively good condition. B.P. was 148/100 mmHg.

Urinalysis

1951. No cystine crystals in the sediment (Laboratory at Karolinska sjukhuset, Stockholm).

1952. Cystine crystals in the sediment (Swedin).

1954. No cystine crystals in the sediment (Persson).

1955. Cystine crystals in the sediment (Persson).

1957 and 1960. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1951. *Gravel washed out from the right renal pelvis that same year*: easily combustible substance which is not uric acid, urate, cystine or amyloid substance (Swedin).

1951. *Calculus passed spontaneously*: cystine stone (Laboratory at Karolinska sjukhuset, Stockholm).

1951 *Calc lus removed from the right renal pelvis that same year* (first operation). cystine stone (Laboratory t Karolinska sjukhuset, Stockholm).

1951 *Calculus passed spontaneously* cystine stone (Laboratory at Karolinska sjukhuset, Stockholm).

1951 *Calculus removed from the right renal pelvis that same year* (second operation): cystine stone (Laboratory at Karolinska sjukhuset, Stockholm).

1951 *Calculus passed spontaneously* cystine stone (Laboratory at Karolinska sjukhuset, Stockholm).

1961 *Urinary calculus (sit and year of removal unknow*). cystine stone (roentgen crystallography Hambraeus & Lagergren).

Data on a Relative of the Above Cystinurics

The paternal grandfather of the 2 cystinurics traced in this family died at the age of 50, the cause of death being given as renal failure and albuminuria.

Family XXXIII

Sixty-four individuals and 3 generations were xamined.

Results. 1 case of homozygous cystinuria. N case of semi-cystinuria.

Case No XXXIII 06.03 S.V N a graduated economist, born in 1921 At the age of 14 (1935) he underwent appendicectomy At the age f 20 (1941) he had his first attack of renal colic, which involved the left side. Radiography revealed calculus in the left ureter from which t was removed by *ureterolithotomy* Thereafter further attacks of renal colic occurred which culminated in the spontaneous passage of gravel At the ge of 26 (1947) he gain had an attack of left renal colic which was particularly severe and ended in the spontaneous passage of calculi. B.P was 140 100 mmHg and serum N.P N 58 mg% Microscopy of the urinary sediment revealed few R B C per high power field and several cystine crystals. There was no albuminuria. During the following two years he had seven attacks of severe left renal colic, each ending in the spontaneous passage of calculi. At the follow-up examination in 1949 there was no evidence of urinary infection. Serum N.P.N was 35 mg% and B.P 170 110 mmHg. There was no albuminuria. Microscopy of the urinary sediment revealed from four to five W.B.C. and numerous R.B.C. per high power field. There was radiographic evidence of the presence of calculi in the left renal pelvis and ureter Later on that same year (1949) he passed 2 calculi spontaneously In 1950 radiographic studies revealed that the calculi in the left kidney had increased in size. B P was 145 95 mmHg and serum N.P.N .5 mg% *Left pyelolithotomy* was performed. Three years later he was admitted to hospital with hypertension, his B.P being then 180/110 mmHg. After a few days bedrest B.P fell to 135 100 mmHg, serum N.P.N being 35 mg% There was no albuminuria. Microscopy of the urinary sediment revealed numerous R.B.C. and W.B C. per high power field and cystine crystals. That same year (1953) *left nephrectomy* was carried out, the indications being hypertension and the presence of left renal calculi. The operation revealed a small kidney and the presence of large calculi in the renal pelvis. Microscopy of the operation specimen disclosed the histological picture of chronic non-specific pyelonephritis. The following year (1954) attacks of right renal colic occurred, one of these being associated with anuria which persisted for two days. His urine was found to contain a trace of albumen. Serum N.P.N was 44 mg% and B.P 160 100 mmHg. A calculus was removed from the right ureter by *ureterolithotomy* The post-operative course was uneventful. H was instructed to maintain high fluid intake. Three years later attacks of right renal colic recurred. There was radiographic evidence of the presence of calculus obstructing the right ureter It was removed by *ureterolithotomy* On admission serum N.P.N was 56 mg% microscopy of the urinary sediment revealed large number of R.B C. per high power field. The post operativ course was uneventful. The following year (1958) there was radiographic evidence of the recurrence of a calculus in the right kidney As the calculus was found to

increase in size it was removed by *pyelolithotomy* (1960), the patient being then 39 years old. B.P was then 135/80 mmHg and serum N.P.N 39 mg%. Two years later (1962) he again had an attack of right renal colic. Radiographic studies revealed normal renal function but also a small calculus in the ureter at the level of the sacroiliac joint which was causing slight ureteric obstruction. The symptoms subsided within 48 hours.

Urinalysis

1941 1947 and 1953 Cystine crystals in the sediment (Laboratory at Karolinska Sjukhuset, Stockholm).

1957 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1941 *Calculus removed from the left ureter that sam year*: cystine stone (Laboratory at Karolinska Sjukhuset, Stockholm).

1947 *Calculus removed from the left ureter that same year*: cystine stone (Laboratory at Karolinska Sjukhuset, Stockholm).

1950: *Calculi passed spontaneously 1 1949* composed of magnesium phosphate and calcium carbonate (Laboratory at Karolinska Sjukhuset, Stockholm).

1950: *Calculus removed from the left renal pel is that same year*: calcium oxalate stone (Laboratory at Karolinska Sjukhuset, Stockholm).

1953 *Calculus removed from the left renal pelvis that same year*: cystine stone (Laboratory at Karolinska Sjukhuset, Stockholm).

1953 *Gravel passed spontaneously in 1953* composed of calcium oxalate and calcium phosphate (Laboratory at Karolinska Sjukhuset, Stockholm).

1954 *Calculus removed from the right ureter that same year*: cystine stone (roentgen crystallography Lagergren).

1957 *Calculus removed from the right ureter that sam year*: calcium oxalate stone (roentgen crystallography Lagergren).

1960: *Calculus removed from the right renal pelvis that same year*: cystine stone (roentgen crystallography Lagergren).

Family XXXIV

Eighteen individuals and 3 generations were examined.

Results: 3 cases of homozygous cystinuria. No case of semi-cystinuria.

Case XXXIV 02 H. K. S., a shop-owner (female), single, born in 1901 She had been healthy until the age of 3 (1933) when she developed diffuse abdominal pain. As appendicitis was suspected ppendicectomy was performed. Six months later she developed pyelitis and passed gravel spontaneously. Radiography revealed bilateral renal calculi. As her symptoms exacerbated she was admitted to hospital (1935). On admission serum N P.N was 39 mg%. There was no albuminuria. Microscopy of the urinary sediment revealed from six to ten R.B.C., from one to eight W.B C per high power field and also bacteria (cocci). There was radiographic evidence of bilateral hydronephrosis and persistence of the bilateral renal calculi revealed by the X-ray examination performed two years previously A calculus, almost the size of a walnut and adhered to the mucosa, was removed from the left renal pelvis by *pyelolithotomy*. After an interval several calculi, the size of peas, were removed from the right renal pelvis by *pyelolithotomy*. Post-operatively serum N.P.N rose to 85 mg% but quickly returned to normal. Thereafter the patient was symptom-free until the age of 59 (1960) when she developed albuminuria and hypertension, B.P being about 250 mmHg systolic. The hypertension quickly subsided with medical treatment. In 1961 radiography disclosed the recurrence of bilateral renal calculi. In 1962 she again had an episode of pyelitis and passed a small calculus spontaneously. B.P was 220/100 mmHg, serum N.P.N 35 mg% and serum creatinine 1.3 mg%. Radiographic examination revealed a calculus in the renal pelvis on either side and left hydronephrosis.

At the follow-up examination in June 1960 the patient' general condition was good, and there were no urinary tract symptoms. B.P was 160/90 mmHg.

Urinalysis

1957 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1935 *Calculus removed from the left renal pelvis that same year:* cystine stone, weighing 9.0 g (Mörner).

1961 *Calculus removed from the left renal pelvis in 1935* composed predominantly of cystine, small amounts of ammonium magnesium phosphate and apatite being present in addition (roentgen crystallography Hambraeus & Lagergren).

Case No XXXIV 04 E. A. L., a house-wife, born in 1905 The patient was married but childless. She had her first attack of renal colic at the age of 23 (1928). B.P was 110/65 mmHg. Her urine was found to contain albumen but no sugar Microscopy of the urinary sediment revealed large number of R.B.C. and several W.B.C. per high power field. Radiography revealed large calculus in the right renal pelvis and it was removed by *pyelolithotomy* Post-operatively B.P was 155/100 mmHg. Thereafter she was virtually symptom-free until the age of 45 (1950) when she again experienced bilateral renal ache off and on.

The patient had a previous history of erysipelas at the age of 26 (1931) scarlet fever associated with albuminuria at the age of 30 (1935), and shingles at the age of 41 (1946).

At the follow-up examination in June 1960 she was found to be in good general condition and had no urinary tract symptoms.

Urinalysis

1957 and 1960 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1935 *Calculus removed from the right renal pelvis in 1928.* oblong cystine stone of light yellowish-grey colour weighing 2.85 g (Mörner).

1961 *Calculus removed from the right renal pelvis in 1928* cystine stone (roentgen crystallography Hambraeus & Lagergren).

Case No XXXIV 06. E. A., a house-wife born in 1911 The patient was married and had one child. At the age of 48 (1959) she had her first attack of renal colic involving the right side. Radiography revealed the presence of a calculus in the left kidney but none in the right.

At the follow-up examination in June 1960 the patient's condition was good and she had no urinary tract symptoms.

Urinalysis

1957 and 1960. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Family XXXV

Six individuals and 2 generations were examined.

Results. 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No XXXV 01 02 E. I. M. S., a ward maid, single, born in 1933 The patient had one child. At the age of 13 (1945) she developed sore throat associated with albuminuria. In 1952 routine examination revealed recurrence of the albuminuria month later she was admitted to hospital with an attack of left renal colic B.P was 150/90 mmHg. Her urine was found to contain a trace of albumen. Microscopy of the urinary sediment revealed from three to five R.B.C. and from five to seven W.B.C. per high power field. Serum N.P.N was 38 mg%. Radiography revealed the presence of bilateral renal calculi. Two calculi were removed from the left renal pelvis by *pyelolithotomy* the operation revealing enlargement of the kidney After an interval of four weeks a coral stone and numerous small calculi were removed from the right renal pelvis by *pyelolithotomy*

In 1953 follow-up examination revealed satisfactory renal function. Serum N.P.N was

24 mg%. Microscopy of the urinary sediment did not reveal any abnormality. Urography demonstrated the presence of calculi in the right kidney. The patient went on a vegetarian diet and remained virtually symptom-free for four years when (1957) she developed haematuria, there being no other urinary tract symptoms. B.P. was 140/90 mmHg. There was albuminuria. Microscopy of the urinary sediment revealed numerous R.B.C. and from eighteen to twenty W.B.C. per high power field, bacteria being absent. Serum N.P.N. was 29 mg% and creatinine clearance 60 ml per minute. Radiography revealed delayed excretion of opaque medium on both sides and bilateral hydronephrosis due to the presence of a large bilateral coral stone. The calculi were removed that same year (1957) by *left pyelolithotomy* followed after four weeks by *right pyelolithotomy*.

The patient's pregnancy (1958) was complicated by pyelitis associated with pyrexia and albuminuria. B.P. was then 115/70 mmHg. Her urine was found to contain albumen but no sugar. Microscopy of the urinary sediment revealed a large number of W.B.C. per high power field and bacteria (rods). Serum N.P.N. was between 26 mg% and 36 mg%. Treatment of the pyelitis with antibiotics was ineffective. Following bilateral ureteric catheterisation her temperature returned to normal. Two months later she had a normal delivery. The following year (1959) pyelitis associated with pyrexia recurred. B.P. was 135/75 mmHg. Her urine was again found to contain albumen but no sugar. Microscopy of the urinary sediment revealed from three to eight R.B.C., a large number of W.B.C. and numerous bacteria (rods) per high power field. Serum N.P.N. was 30 mg%. Radiography revealed the recurrence of calculi in the renal pelvis on either side and the presence of a calculus obstructing the left ureter which was removed by *ureterolithotomy*. The urinary infection was treated with antibiotics. The post-operative course was uneventful.

In 1960 she was re-admitted to hospital for follow-up examination. There still was radiographic evidence of bilateral renal calculi. Serum N.P.N. was 30 mg% and B.P. 135/70 mmHg. Apart from occasional haematuria she had had no other urinary tract symptoms since her previous stay in hospital (1959). Nevertheless it was decided to operate upon her removing 1 large and 1 small calculus from the right renal pelvis by *pyelolithotomy* and *partial nephrectomy*. Histological examination of the operation specimen revealed the picture of chronic, non-specific pyelonephritis. Post-operatively serum N.P.N. rose to 50 mg% but quickly returned to normal. She was put on a high fluid intake and sodium bicarbonate and went on a vegetarian diet of her own free will. Post-operatively there was still radiographic evidence of bilateral renal calculi.

Urinalysis

1957 and 1960: Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

1953: *Calculus removed from the left renal pelvis in 1952*, cystine stone (Norman).

1953: *Calculus removed from the right renal pelvis that same year*, composed predominantly of cystine, a trace of calcium oxalate being present in addition (Tryding).

1961: *Urinary calculus (site unknown)*, composed predominantly of cystine, small amounts of apatite and ammonium magnesium phosphate being present in addition (roentgen crystallography: Hambraeus & Lagergren).

1961: *Calculus removed from the right renal pelvis in 1960*, composed predominantly of ammonium magnesium phosphate and apatite, a trace of cystine being present in addition (roentgen crystallography: Hambraeus & Lagergren).

Family XXXVI

Seven individuals and 3 generations were examined.

Results. 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XXXVI 01 01. C. A. A., a managing director, born in 1912. The patient had his first attack of renal colic culminating in the

spontaneous passage of several calculi at the age of 26 (1938). Thereafter he had no urinary tract symptoms until the ge of 3 (1944) when a similar attack occurred ending in the spon taneous passage of several calculi. This was followed by several further attacks of renal colic associated with macroscopic haematuria. In 1950 he had a severe attack of right renal colic. B.P was 140/90 mmHg. As there was radiographic evidence of non-function of the right kidney and the presence of a coral stone therein, *pyelolithotomy* and *ureterolithotomy* were performed, the coral stone and several small calculi being removed from the renal pelvis, and several small calculi from the distal ureter The renal pelvis was found to be moderately enlarged at operation. Post-opera tively the patient was symptom-free for about two months. Then he developed pyelitis as sociated with pyrexia and had several attacks of bilateral renal colic culminating in the spon taneous passage of calculi. Radiography revealed normal function of both kidneys but dilatation of the right renal pelvis and the presence of a calculus in the right ureter Two weeks later he again had a severe attack of right renal colic associated with pyrexia (39 C) and macroscopic haematuria. As there still was radiographic evidence of non-function of the right kidney *ureterolithotomy* was once more performed, the operation evealing dilatation of both the renal pelvis and ureter The post operative course was complicated by pyrexia. Following treatment with antibiotics the temperature returned to normal. In 1955 further attacks of renal colic occurred which did not require treatment in hospital. Thereafter he often had renal ache and there was tenderness in the region of the kidneys.

At the age of 23 (1935) the patient was admitted to hospital with gastritis. In 1955 he was treated for pulmonary tuberculosis.

Urinalysis

1957 Abnormal amounts f cystine, lysine arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1950 *Calculus removed from the right renal pelvis that same year* cystine stone (G. Hammarsten).

1951 *Calculus passed spontaneously* cystine stone (G Hammarsten).

Family XXXVII

Ten individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. 3 cases of semi-cystinuria.

Case No XXXVII 01 01 E. V L., a fisher man, born in 1913 At the age of 28 (1941) he developed abdominal pain and was admitted to hospital with suspected gastroenteritis. Nine years later (1950) abdominal pain recurred. Excretion urography revealed delayed excre tion of opaque medium on the left side. During 1952 he was admitted to hospital seven times with acute abdominal pain. I order to investigate the cause of the latter exploratory laparotomy was carried out but no abnormality was found. Post-operatively he developed ventral hernia. On one occasion there was microscopic haematuria. Urography revealed fairly large calculus in the left renal pelvis from which it was removed by *pyelolithotomy* The following year (1953) bdominal pain re curred and he was again admitted to hospital several times for investigation of its cause. On one of these occasions his ventral hernia was surgically repaired. After thorough medical check-up of the patient that same year (1953) the diagnosis of duodenal ulceration was made and a Billroth II type partial gastrectomy was performed. Shortly before his admission to psychiatric clinic, to which he had been refer red on account of dementia simplex, chronic alcoholism, and narcolepsy he had further attack of abdominal pain associated with mic roscopic haematuria Radiography evealed a calculus, the size of hazel-nut, in the right renal pelvis from which it was removed by *py lolithotomy* (1955). Thereafter he repeatedly attended hospital complaining of excruciating renal ache. Occasionally microscopic haematuria was found, but as rule he was considered to be malingering.

At follow-up examination in 1957 he still complained of renal ache but his general condi tion was found to be good. B.P was then 130/70 mmHg. Microscopy of the urinary sedi-

ment revealed a large number of cystine crystals per high power field. Serum N.P.N and creatinine clearance were normal. Radiography revealed a renal calculus on the left side.

The patient died in a drowning accident at the age of 46 (1959).

Urinalysis

1953 Cystine crystals in the sediment (Laboratory at the Hospital in Varberg).

1957 Abnormal amounts of cystine, lysine arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1952. *Calculus removed from the left renal pelvis that sam year* cystine stone (Laboratory at Sahlgrenska Sjukhuset, Göteborg).

This case has previously been reported by Lauritzen (1957).

Family XXXVIII

Sixteen individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XXXVIII 02 04 H O R., a student (male), born in 1940. At the age of 8 years (1948) he had frequency of micturition and dysuria for two weeks. A vesical calculus was removed by *suprapubic cystolithotomy* Radiography revealed non-functioning hydronephrotic kidney on the left side. Three years later (1951) he developed abdominal pain on the right side. The presumptive diagnosis of appendicitis was made and appendicectomy was carried out. Post-operatively there still was *radiographic evidence of no excretion of* opaque medium on the left side and abdominal pain persisted being associated with macroscopic haematuria. Following sulpha drug therapy he developed anuria associated with a rise of serum N P.N to 150 mg% He was put on a high fluid intake and sodium bicarbonate The anuria subsided and serum N.P.N returned to normal. Radiography still revealed non-function of the left kidney and there was evidence of the presence of 1 large and several small calculi in the right renal pelvis, the *calculi being removed by pyelolithotomy* (1952). As serum N.P.N was between 24 mg% and 32 mg% every effort was made to keep his urine alkaline. Thereafter he was several times admitted to hospital with attacks of renal colic or for follow-up examination of his renal function. Serum N.P.N was between 24 mg% and 39 mg% Radiography performed shortly after the pyelolithotomy carried out in 1952, revealed the recurrence of calculi in the right kidney Follow-up X-ray examinations showed that the calculi were increasing in size. The following year (1953) he developed anuria and this made a second operation imperative, the calculi being removed by *pyelo-ureterolithotomy* Post-operatively he passed several calculi, the size of grains of rice, spontaneously Serum N.P.N was between 29 mg% and 45 mg%

The patient thereafter attended hospital regularly for follow-up examinations. In 1954 there was gain radiographic evidence of the recurrence of calculi in the right renal pelvis. He was put on a high fluid intake and was given sodium citrate to maintain his urine alkaline. In 1955 he had a further attack of renal colic which culminated in the spontaneous passage of calculi and was associated with rise in serum N.P.N to 59 mg% Thereafter he had off and on further attacks of renal colic with occasional spontaneous passage of calculi. There was radiographic evidence that the calculus in the right renal pelvis was increasing in size and in 1956 radiography revealed it to be a coral stone several smaller calculi being demonstrated in addition in the right renal pelvis. The calculi were removed by *pyelolithotomy* Serum N P.N was between 44 mg% and 33 mg% *Thereafter he was once more put* on high fluid intake (about 3 litres per 4 hours) and sodium bicarbonate. He reported twice yearly for follow-up examinations which revealed the recurrence of small calculus in the right kidney which had not been increasing in size during the past few years. Apart from occasional haematuria following vigorous exercise (g tennis match) he had no urinary tract symptoms. H was exempt from active military service.

At the follow-up examination in May 1960 the patient was found to be in good general condition, there being no urinary tract symptoms. B.P was 125/90 mmHg.

Urinalysis

1957 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1948 *Calculus removed from the bladder that same year*: cystine stone (Lehman).

1952. *Calculus removed from the right renal pelvis that same year*: cystine stone (Laboratory at the Hospital in Lund).

1953 *Calculus removed from the right ureter that same year*: cystine stone (Jacobsson).

1955 *Calculus passed spontaneously*: cystine stone (Hansson).

1956: *Calculus removed from the right renal pelvis that same year*: composed of cystine calcium magnesium phosphate, and traces of calcium oxalate (Hansson).

1961 *Calculus removed from the right renal pelvis in 1952*: composed predominantly of cystine small amounts of patite being present in addition (roentgen crystallography Hambraeus & Lagergren).

Family XXXIX

Twenty-one indi iduals and 4 generations were examined.

Results: 1 case of homozygous cystinuria (screening case)
6 cases of semi-cystinuria.

Case No XXXIX 01 02 02 A. C. N school-girl, born in 1950. At the ge of 6 (1956) she had an upper respiratory infection associated with cystitis, there being no other urinary tract symptoms.

At the follow-up examination in September 1960, she was found to be in good general condition. The heart, lungs, and abdomen did not show any bnormality B.P was 140 70 mmHg.

Urinalysis

1957 and 1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Family XL

Fourteen individuals and 3 generations were examined.

Results: 2 cases of homozygous cystinuria (screening cases).
No case of semi-cystinuria.

Case No XL 01 01 01 K. O I a schoolboy born in 1950. He had no urinary tract symptoms.

Urinalysis

1957 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Case No XL 01 01 0 K. B. I a school girl, born in 1951 She had no urinary tract symptoms.

Urinalysis

1957 Abnormal amounts of cystine lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

N stones available.

Family XLI

Twenty individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria.
No case of semi-cystinuria.

Case No. XLI 01 01 01 M G A. S., an office clerk (female), single, born in 1939 From the age of 9 (1948) she had had repeated episodes of pneumonia. At the age of 14 (1953) she underwent appendicectomy and the fol

lowing year (1954) cholecystectomy She also had a persistent ductus arteriosus (Botalli) which was operated on when she was 17 (1956). Six months after the operation it was found that the pulmonary hypertension was still present; there was clinical evidence of an atrial septum defect the picture resembling Eisenmenger's syndrome. Although the patient's capacity for work was greatly reduced she was fit enough to do an office clerk's work.

From the age of 19 (1958) she had had several episodes of cystitis associated with bilateral renal ache particularly in the right side. Radiography performed two years later (1960), revealed delayed excretion of opaque medium on the right side, associated with dilatation of the renal pelvis and ureter and the presence of 2 calculi in the renal pelvis on this side. Her urine contained a trace of albumen but no sugar Microscopy of the urinary sediment revealed between five and ten R.B.C. and numerous W.B.C. per high power field. Penicillin therapy resulted in an improvement in her condition.

Thereafter she had occasional episodes of cystitis. Two years later (1962) she developed pyelocystitis associated with pyrexia which was successfully treated with streptomycin. Her urine was found to contain albumen but no sugar Microscopy of the urinary sediment revealed numerous W.B.C., between two and four R.B.C. and a few bacteria (rods) per high power field. B P was 170 85 mmHg. Serum N.P.N was 30 mg% Radiography (1962) revealed that the calculi demonstrated in 1960 were still present an additional finding being a calculus in the left renal pelvis. As the patient cardiac lesion contra-indicated any renal operation, her urinary tract symptoms were treated medically

Urinalysis

1958 and 1960 Abnormal amounts of cystine lysine arginine and ornithine (Boström & Hambraeus).

Stone analysis

No stones available

Family XLII

Twelve individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. N case of semi-cystinuria.

Case No. XLII 01 04 P T G O., an electrician, born in 1927 The patient had enjoyed good health until the age of 25 (1952) when he began to have attacks of renal colic which culminated in the spontaneous passage of calculi and were occasionally associated with haematuria. Excretion urography (1955) revealed hydronephrosis and delayed excretion of opaque medium on the right side and the presence of a calculus in the right ureter from which it was *removed endoscopically* The following year (1956) he was admitted to hospital with an attack of left renal colic which subsided spontaneously Serum calcium was 11.4 mg% and serum phosphorus 3.7 mg% In 1957 a further attack of renal colic occurred, involving the right side B.P was 140 80 mmHg. Serum calcium was 10.8 mg% Excretion urography revealed a calculus in the right renal pelvis and delayed excretion of opaque medium on this side. Microscopy of the urinary sediment revealed numerous R.B.C. per high power field. Four weeks later an attack of right renal colic recurred. Excretion urography revealed no excretion of opaque medium on the right side and 3 calculi in the right ureter which were removed by *ureterolithotomy* Thereafter he had occasional attacks of renal colic. In 1959 he had a further attack of right renal colic which was particularly severe Microscopy of the urinary sediment revealed numerous R.B.C. per high power field. He eventually passed 2 calculi, the size of peas, spontaneously Thereafter he had attacks of renal colic off and on culminating in the spontaneous passage of calculi.

At the follow-up examination in May 1960 his general condition was found to be good but he still had attacks of renal colic off and on.

Urinalysis

1960: Abnormal amounts of cystine lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

1959: *Calculus passed spontaneously*: cystine stone (O. Hammarsten).

1961: *Calculi passed spontaneously*: composed predominantly of cystine, a small quantity of apatite being present in addition (roentgen crystallography: Hambraeus & Lagergren).

Family XLIII

Nine individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XLIII 01 03: S. B. N., a building engineer born in 1931. The patient enjoyed good health until the age of 20 (1951) when he passed gravel spontaneously on one occasion. At the age of 28 (1959) he had his first attack of renal colic which involved the left side and was associated with pyrexia. His urine was found to contain a trace of albumen but no sugar. Microscopy of the urinary sediment revealed numerous R.B.C. and W.B.C. per high power field. B.P. was 150/80 mmHg. Radiography revealed the presence of a large calculus in the right renal pelvis and a calculus in the left ureter which was causing obstruction and which was *removed endoscopically*. Post-operatively he passed a calculus, the size of a pea, spontaneously. The urinary tract infection was controlled with antibiotics. Serum N.P.N. was between 33 mg% and 40 mg%.

The patient attended hospital regularly for follow-up examinations. At the age of 29 (1960) he was once more admitted to hospital with pyelitis. B.P. was then 130/80 mmHg. Microscopy of the urinary sediment revealed between twenty and twenty-five R.B.C. and numerous W.B.C. per high power field. Radiography revealed the presence of bilateral renal calculi. A large coral stone on the right side was removed by *pyelolithotomy*. Post-operatively urine leaked from the wound but this leakage eventually ceased spontaneously.

After his discharge home he developed pyrexia. He was therefore once more admitted to hospital and again given antibiotics. Thereafter he remained virtually symptom-free but in 1962 radiography revealed that the calculus in the left renal pelvis had increased in size. *Pyelolithotomy* was once more performed, this calculus, which was the size of a pigeon's egg, and several smaller calculi being removed from the left renal pelvis. The post-operative course was complicated by pyrexia and he was again given antibiotics.

At the follow-up examination in June 1960 his general condition was found to be good. B.P. was 130/90 mmHg.

Urinalysis

1959: Cystine crystals in the sediment (Tottie–Olson).

1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1961: *Calculi passed spontaneously*: composed predominantly of cystine, a very small amount of apatite being present in addition (roentgen crystallography: Hambraeus & Lagergren).

Family XLIV

Twenty-three individuals and 4 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XLIV 03 02: A. S. L., a primary school-teacher (male), born in 1933. At the age of 3 he scalded his left arm and foot. In 1954 he underwent appendicectomy. Two years later (1956) he was admitted to hospital with abdominal pain. He had his first attack of renal colic at the age of 26 (1959). This was followed by repeated attacks of bilateral renal colic. Radiography (1959) revealed bilateral renal calculi but no ureteric obstruction. The following year (1960) he passed the left renal calculus spontaneously; as the right one was found to be increasing in size it was removed by *pyelolithotomy* that same year (1960). B.P. was 125/80 mmHg. There was no albuminuria. Microscopy of the urinary sediment revealed between fifteen and twenty R.B.C. and between ten and fifteen W.B.C. per high power field.

Post-operatively there was pyrexia and bac teriuria. He was therefore given sulpha drugs. Serum N.P.N was 29 mg% Thereafter he remained virtually symptom-free. When he was 29 (1962) diabetes was diagnosed.

At the follow-up examination in May 1960 his general condition was found to be good and he had no urinary tract symptoms.

Urinalysis

1960 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1960 *Calculus passed spontaneously* composed predominantly of cystine, calcium oxalate being present in the peripheral parts (roentgen crystallography Hambraeus & Lager gren).

1961 *Calculus removed from the right renal pelvis in 1960* composed predominantly of cystine small amounts of calcium oxalate being present in addition (roentgen crystallography Hambraeus & Lagergren).

Family XLV

Four individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XLV 01 03 B B. L., correspondence clerk (male), born in 1925 As a child he had measles, rubella, and mumps. At the ages of 3 (1928) and 6 (1931) he had an upper respiratory infection complicated by albuminuria.

At the age of 14 (1939) urinary tract symptoms commenced in the form of severe abdominal pain in the left side, the pain resembling renal colic and being associated with pyrexia. There was albuminuria. Roentgenography revealed the presence of a calculus in the bladder and bilateral hydro-ureter As there was dysuria the calculus was removed from the bladder by *suprapubic cystolithotomy* Six years later (1945) there was radiographic evidence of the presence of a calculus in the right kidney and the following yea (1946) also of one n the left kidney He continued to ha e attacks of renal colic and episodes of pyelocystitis. At the age of 25 (1950) radiographic studies revealed that the calculus on the left side was causing ureteric obstruction, it was therefore removed by *ureterolithotomy* The following year (1951) the calculus in the right kidney was removed by combined *pyelolithotomy* and *partial nephrectomy* The kid ney was found to be abnormally small and to show pyelonephritic change. Four months later a further attack of left renal colic occurred and radiography revealed the recurrence of a calculus in the left ureter Serum calcium was 12.5 mg% and serum uric acid 4 4 mg% Microscopy of the urinary sediment revealed a large number of W.B.C. per high power field. Later that same year (1951) he had a further episode of cystitis which was associated with macroscopic haematuria and al buminuria. The cystitis eventually subsided. Two years later (1954) he had further attack of right renal colic associated with pyrexia. Radiography then revealed a calculus in the right ureter causing ureteric obstruction. *Pyrexia subsided during treatment with antibiotics.* Serum N.P.N was between 25 mg% and 33 mg% His urine was found to be acid. Microscopy of the urinary sediment revealed numerous W.B C. and R.B.C. per high power field. Two months later he had a further attack of renal colic associated with cystopyelitis. Serum N.P.N was between 29 mg% and 46 mg% B.P was 150/110 mmHg. *Endoscopic removal* of the right ureteric calculus was attempted but only gravel was obtained. He had a further attack of right renal colic and as the calculus could not be removed endoscopically *ureterolithotomy* was performed, the operation revealing the calculus to be the size of a bean. Post-operatively serum N.P.N was between 37 mg% and 50 mg% but soon returned to normal. B.P was 160/110 mmHg. However attacks of renal colic continued to occur and he developed frequency of micturi tion in addition. In 1958 he was admitted to hospital with suspected hyperparathyreoidism. Radiographic studies carried out during hi stay in hospital revealed normal excretion of opaque medium on both sides but evidence of a calculus in the right renal pelvis. *Pyelolitho-*

tomy combined with *partial nephrectomy* was again performed. Apart from a short-lived pyrexia the post-operative course was uneventful. Serum calcium was then between 11 mg% and 11.6 mg%. The urinary tract infection subsided and he was discharged home symptom-free.

Thereafter he remained well for about a year when a further attack of *left renal colic* associated with microscopic haematuria occurred, there being no symptoms of urinary tract infection. Urography did not reveal any abnormality. Four months later that same year (1959) radiography revealed the recurrence of a calculus in the left renal pelvis; it was removed by *pyelolithotomy*. Serum calcium was between 8.4 mg% and 10.4 mg%. Serum N.P.N. was between 76 mg% and 44 mg% and serum phosphorus between 9.4 mg% and 12.5 mg%. Post-operatively he had an attack of short-lived pyelitis. Four weeks later he attended hospital for *follow-up* examination and was found to be virtually symptom-free. B.P. was 150/80 mmHg. Serum N.P.N. was 28 mg%, serum calcium 9.6 mg% and serum phosphorus 11.9 mg%.

At the follow-up examination in May 1960, his general condition was found to be good but he had attacks of renal colic off and on.

Urinalysis

1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1951. *Calculus removed from the right renal pelvis that same year*: composed of fibrin and small amounts of calcium ammonium phosphate (Laboratory at the Hospital in Lund).

1954. *Calculus removed from the right ureter that same year*: composed of calcium ammonium phosphate and cystine, urate and oxalate being absent (Ekstrom).

1958. *Calculus removed from the right renal pelvis that same year*: composed of calcium oxalate and phosphate (Arvidson).

1959. *Calculus removed from the left renal pelvis that same year*: cystine stone (Laboratory at the Hospital in Malmö).

Family XLVI

Two individuals and generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XLVI 91. S.D., a keeper at a Zoo, born in 1931. The patient had remained well until the age of 22 (1953) when he began to have attacks of bilateral renal colic which were associated with haematuria and culminated in the spontaneous passage of calculi. Radiographic studies (1958) revealed the kidneys to be normal in size and function and there was no ureteric obstruction. There was no albuminuria. Serum N.P.N. was 38 mg% and serum calcium 9.8 mg. Two months later a further attack of renal colic associated with haematuria occurred. Radiography revealed the presence of a calculus in the right ureter. There was albuminuria. Serum N.P.N. was between 44 mg% and 45 mg%. That same year (1958) combined right *pyelolithotomy* and *partial nephrectomy* were performed, 3 calculi being removed from the renal pelvis. Thereafter he had no urinary tract symptoms for about a year when he had a further attack of right renal colic. Radiography this time did not reveal any abnormality. This latter attack was followed by a severe attack of left renal colic. B.P. was 150/100 mmHg. Radiographic studies revealed a calculus in the left renal pelvis which was causing obstruction and was removed by *pyelolithotomy* (1960). The post-operative course was uneventful. Two weeks after this operation he had an attack of right renal colic. Microscopy of the urinary sediment did not reveal any abnormality. There was albuminuria. Serum N.P.N. was 50 mg%. B.P. was 140/80 mmHg. Radiography revealed a calculus in the right ureter which was causing obstruction. There was no urinary tract infection. A third right *ureterolithotomy* (1960) was performed. Two months later a severe attack of left renal colic recurred. Radiography revealed the recurrence of a large calculus in the left ureter, which was causing obstruction, and the presence of several smaller calculi in the right renal pelvis. There also was

pyelitis. As all these symptoms subsided he was not operated upon.

At the follow-up examination in May 1960 the patient's general condition was found to be good and he had no urinary tract symptoms.

Urinalysis

1960: Abnormal amounts of cystine, lysine arginine and ornithine (Boström & Hambraeus).

Stone analysis

1958. *Calculus removed from the right renal pelvis that same year:* cystine stone, there being no inorganic elements present (Arwidson).

1960: *Calculus removed from the left renal pelvis that same year:* cystine stone (Tryding).

1960. *Calculus removed from the right ureter that same year:* cystine stone (Laboratory at the Hospital in Lund).

Family XLVII

Six individuals and 3 generations were examined.

Results: 3 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No. XLVII 01 01 S G L G., a GPO workman, born in 1928. The patient had his first attack of renal colic at the age of 23 (1951): it involved the right side. Radiographic studies revealed a calculus, the size of a pea on the right side which was causing ureteric obstruction. B.P was 120/85 mmHg. Two days later he passed 2 small crystalline calculi spontaneously. The following year (1952) he had an attack of left renal colic. He had never had either haematuria or albuminuria.

At the age of 24 (1952) he was for seven months in hospital on account of tuberculosis of the right lung. On discharge home he was symptom-free.

Urinalysis

1959: Abnormal amounts of cystine (Vikblad).

1960: Abnormal amounts of cystine lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Case No. XLVII 01 02 S. A. O G a fisherman, born in 1930. At the age of 7 (1937) the patient had symptoms suggestive of encephalitis. From the age of 16 (1946) he had symptoms of peptic ulceration and was nervous. At the age of 20 (1950) radiography revealed signs of gastritis but not of peptic ulceration. Microscopy of the urinary sediment performed that same year revealed numerous bacteria and W.B.C. per high power field. Treatment with antibiotics resulted in controlling his urinary tract infection. Radiography demonstrated a coral stone in the left kidney Serum N.P.N was between 35 mg% and 42 mg%. Urography performed the following year (1951) demonstrated almost complete non-function of the left kidney Nephrectomy was therefore carried out. The kidney showed cystic degeneration, only a thin rim of renal parenchyma remaining, and the renal pelvis contained a coral stone with a rough surface, twice the size of a walnut. Histologic examination of the kidney was not performed. Thereafter the patient was symptom-free

Urinalysis

1959: Abnormal amounts of cystine (Vikblad).

1960: Abnormal amounts of cystine lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

Not performed.

Case No XLVII 01 04 R. G an electrician, born in 1940. When the patient was 18 years old (1958) an attack of left renal colic associated with anuria and a rise in serum N.P.N to 92 mg% occurred. Radiography revealed enlargement of the left kidney and a probable stone in the left ureter: the right kidney was not outlined. On the basis of these findings left *ureterolithotomy* was performed (1958). The post-operative course was uneventful. Serum N.P.N fell to 39 mg% in less than a week The following year (1959) X-ray examination confirmed the presumptive diagnosis of agenesis of the right kidney which

had been made in 1958. Three months later that same year a further attack of renal colic occurred which was associated with pyrexia but subsided quickly B.P was 130/90 mmHg. After a further three months the patient experienced abdominal pain on the right side. As the pain increased in severity appendicitis was suspected and appendicectomy was performed, the operation revealing an appendix abscess. The post-operative course was uneventful. Urography (1960) did not reveal any abnormality of the left kidney but the patient had had one episode of pyelocystitis. Three months later he had a further attack of left renal colic. Microscopy of the urinary sediment revealed several R.B.C. and numerous W.B.C. per high power field. There was tenderness in the region of the left kidney He developed anuria which was caused by a calculus which had got held up at the pelviureteric junction. *Pyelolithotomy* was therefore performed, one large and several small calculi being removed from the left renal pelvis. The post-operative course was uneventful. Creatinine clearance was 92 ml per minute. Eighteen months later he was once more admitted to hospital with urinary tract infection which subsided quickly following treatment with antibiotics. B.P was then 145 75 mmHg. Microscopy of the urinary sediment revealed few R.B.C and W.B.C per high power field Serum N P.N was 28 mg% and creatinine clearance 86 ml per minute.

Urinalysis

1959· Abnormal amounts of cystine (Vikblad).

1960· Abnormal amounts of cystine lysine arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1958 *Calculus removed from the left ureter that same year*· cystine stone (Vikblad).

Family XLVIII

Sixteen individuals and 3 generations were examined.

Result 4 cases of homozygous cystinuria. No case of semi-cystinuria

Case No XLVIII 01 03 N A., a female patient, single, born in 1908 At the age of 20 (1928) the patient developed left renal ache associated with pyrexia which lasted about two weeks. Thereafter she experienced occasional stabbing pain in that region. At the age of 41 (1948) the pain became increasingly severe and was associated with frequency of micturition. The urine was found to contain albumen but no sugar Microscopy of the urinary sediment revealed a large number of W.B C. per high power field. B.P was 165/95 mmHg. Serum N.P.N was 31 mg% As radiography revealed deformity and virtual non-function of the left kidney *nephrectomy* was carried out. The operation revealed a complete, degenerated kidney which contained cavities filled with pus, only thin rim of the renal parenchyma remaining. The post-operative course was uneventful. Thereafter she occasionally experienced right renal ache. Radiography (195) revealed a calculus in the right kidney there being no other abnormality As the renal calculus was observed to be increasing in size and as the right renal ache persisted the calculus was removed by *pyelolithotomy* (1953), the operation revealing that it almost completely filled the renal pelvis. Serum N.P.N was 33 mg% B.P was 125 80 mmHg. Serum calcium was between 10 3 mg% and 11.2 mg% Microscopy of the urinary sediment revealed numerous W.B.C. and one or two R.B C. per high power field. A post-operative X-ray examination revealed the presence of calculi and gravel in the renal pelvis. Apart from occasional episodes of urinary infection the patient was symptom-free for the next six years.

In 1959 she was admitted to hospital with abdominal pain on the right side which resembled renal colic, *pyelitis* and *eventually* also anuria which was associated with a rise in serum N P N to 64 mg Catheterisation of the ureter was therefore performed. Radiography revealed compensatory enlargement of the right kidney and the presence of multiple calculi therein Creatinine clearance was then 69 ml per minute The pain in the right side subsided. The patient regularly attended hospital for follow-up examinations and creatinine clearance was 74 ml per minute. Right *pyelo-*

lithotomy was performed later that same year (1959), 6 calculi being removed from the right renal pelvis. Post-operatively serum N.P.N. rose to 57 mg%. The urinary tract infection was treated with antibiotics.

Urinalysis

1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1953: *Calculus removed from the right renal pelvis that same year*: calcium oxalate stone (Laboratory at the Hospital in Malmö).

1959: *Calculus removed from the right renal pelvis that same year*: cystine stone (Laboratory at the Hospital in Malmö).

Case No. XLVIII 01 04. S. H. A., a plumber born in 1912. The patient had his first attack of renal colic at the age of 22 (1934). It involved the right side and culminated in the passage of gravel. Eight years later (1942) further attack of right renal colic occurred. Thereafter there were no urinary tract symptoms other than occasional episodes of mild cystitis until 1958 when an attack of right renal colic recurred. B.P. was then 190/80 mmHg. Radiography revealed the presence of a calculus in the right ureter which was causing obstruction, but twenty-four hours later the patient was symptom-free.

From the age of 27 (1939) the patient had been suffering from peptic ulceration in addition. Six years later (1945) he was admitted to hospital for medical treatment of the lesion. As this form of therapy was ineffective and radiography performed nine years later (1954) revealed pyloric stenosis a Billroth II type partial gastrectomy was performed. Thereafter he was free from peptic ulcer symptoms.

Urinalysis

1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Case No. XLVIII 01 07. T. F. A., a male patient born in 1921. He had no urinary tract symptoms.

Urinalysis

1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Case No. XLVIII 01 09. B. E. A., a male patient, born in 1925. He had no urinary tract symptom, but at the age of 35 (1960) and again at 37 (1962) he had symptoms of peptic ulceration.

Urinalysis

1960: Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Family XLIX

Three individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. XLIX 01 02. G. V. B., a mechanic, single, born in 1917. As a child he had several episodes of bilateral otitis media. At the age of 14 (1931) he had aseptic meningitis. At the age of 16 (1933) he developed right-sided abdominal pain which was thought to be due to appendicitis and appendicectomy was therefore performed. From 1942 he had been suffering from epilepsy and was accordingly taking 0.1 g phenobarbital daily. At the age of 17 he had his first attack of renal colic. It involved both sides and culminated in the spontaneous passage of calculi. The following year (1935) a calculus was removed from the left renal pelvis by *pyelolithotomy*. Shortly after the operation he passed a calculus from the right kidney spontaneously. Thereafter he was more or less symptom-free until the age

of 33 (1950) when he passed calculi spontaneously without experiencing any noteworthy discomfort. This was followed by repeated attacks of renal colic ending in the spontaneous passage of calculi. At the age of 40 (1957) he was admitted to hospital with hypertension (B.P 235/140 mmHg) and albuminuria. Serum N.P.N was 47 mg% Creatinine clearance was 50 ml per minute. Thereafter he passed gravel, there being occasionally renal ache haematuria, and dysuria. These symptoms persisted for several weeks. The patient's condition deteriorated and he often felt nausea. Serum N.P.N was between 50 mg% and 57 mg% In 1959 he was admitted to hospital for renal function tests. B.P was then 260/150 mmHg. Serum N.P.N was 56 mg% Creatinine clearance was 22 ml per minute. Serum calcium was 10 4 mg% and serum phosphorus 4.0 mg% From 1959 he regularly attended hospital for follow-up examinations, serum N.P.N remaining between 50 mg% and 60 mg%

When he was 42 years old (1959) he developed mild heart failur and glycosuria. From 1959 he had been unfit for work owing to his renal and cardiac trouble. He died three years later (1962) at the age of 45 the causes of death being given as hypertension, cardiac failure and chronic pyelonephritis.

Urinalysis

1935 Cystine crystals in the sediment (Laboratory at the Hospital in Västerås).

1960 Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambræus).

Stone analysis

1935 *Calculus removed from the left renal pelvis that same year* cystine stone (Mörner).

1961 *Calculus removed from th left renal pel is 1935* composed predominantly of cystine, very small amount of apatite being present in addition (roentgen crystallography Hambræus & Lagergren).

Family L

Seven individuals and 3 generations were examined.

Results 2 cases of homozygous cystinuria. No case of semi-cystinuria.

Case N L.01 02 S L.B a house-wife born in 1935 The patient was married and had one child. At the age of 16 (1951) she had her first attack of renal colic it was severe in character and ended in the passage f gravel Three years later (1954) a further attack of left renal colic occurred. Radiography revealed the presence of a calculus in the left ureter from which it was removed that same year by *ureterolithotomy* Post-operatively she passed a small calculus spontaneously Thereafter she was repeatedly admitted to hospital with ttacks of left renal colic. At the age of 21 (1956) the radiographic appearances suggested the presence of a bilateral duplex renal pelvis there being no other bnormality shown. Serum calcium was 9 1 mg% Thereafter she had a further attack of renal colic culminating in the spontaneous passage of calculi. At the age f 25 (1960) radiography revealed the presence of a calculus in the left ureter Serum calcium was between 9.8 mg% and 10 1 mg% Serum phosphorus was between 4.0 mg% and 3.5 mg% and serum creatinine 1.0 mg% She passed the ureteric calculus spontaneously Thereafter the patient was more or less symptom-free.

Urinalysis

1960. Abnormal amounts of cystine, lysine, arginine, and ornithine (Bostrom & Hambræus).

Stone analysis

1960 Calculus removed from the left ureter in 1954 cystine stone traces of calcium oxalate being present in ddition (Odin).

1961 *Calculus passed spontaneously* composed predominantly of cystine, traces of apatite being present in addition (roentgen crystallography Hambræus & Lagergren).

Case No. L.01 03 A.B h., male patient, born in 1943 He had no urinary tract symptoms.

Urinalysis

1960 Abnormal amounts of cystine, lysine arginine, and ornithine (Bostrom & Hambræus).

Stone analysis

No stones available.

Family LI

Thirteen individuals and 4 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. LI 01 04 01 N U S. M an engineer born in 1913 At the age of 21 (1934) the patient underwent appendicectomy At the age of 24 (1937) he had his first attack of renal colic; it involved the left side and culminated in the spontaneous passage of calculi. Radiography performed after this attack did not reveal the presence of any further renal calculi. Thereafter he remained well until 1941 when attacks of renal colic ending in the spontaneous passage of calculi recurred at from four to five years interval being occasionally associated with haematuria. Radiographic studies performed in 1958 revealed the presence of bilateral renal calculi, the stone in the left renal pelvis being the size of a walnut. Renal function was normal. Examination of the patient three years later (1961) *revealed that the left renal calculus* had markedly increased in size. Microscopy of the urinary sediment revealed a large number of cystine crystals per high power field. B.P was 155/75 mmHg. Serum N.P.N was 48 mg% and creatinine clearance 82 ml per minute. PAH-clearance was 466 ml and 388 ml per minute. A few months later that same year the calculus was removed from the left renal pelvis by *pyelolithotomy* The post operative course was uneventful. Radiography performed after the operation demonstrated small bilateral renal calculi. Thereafter the patient remained more or less symptom-free, but had been *drinking heavily for several years* and had repeatedly been treated in a clinic for chronic alcoholica.

At the follow-up examination in 1962 he stated that he had no noteworthy urinary tract symptoms; creatinine clearance was then 140 ml per minute.

Urinalysis

1958 and 1961 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1961 *Calculus removed from the left renal pelvis that same year*: cystine stone (roentgen crystallography Hambraeus & Lagergren).

Family LII

Fifty individuals and 3 generations were examined.

Results: 2 cases of homozygous cystinuria. No case of semi-cystinuria.

Case No LII 03 06 H. R. I. O a breeder of furred animals, born in 1924 When the patient was 24 years old (1948) urinary tract symptoms commenced and the following year (1949) a right renal calculus was removed by *nephrectomy* The operation specimen revealed calculus in the renal pelvis and pyonephrosis' only a thin rim of the renal parenchyma remained. The urinary tract infection was treated with an acidogenic drug. Post-operatively the patient had an attack of left renal colic. The following year (1950) similar attack recurred. *Radiography revealed a calculus in the left* renal pelvis from which it was removed by *pyelolithotomy* The post-operative course was uneventful. Thereafter the patient passed calculi spontaneously off and on and regularly attended hospital on account of cystitis. Urography in 1951 did not reveal any abnormality Serum N.P.N was 40 mg% In 1953 he had further attack of left renal colic and was once more admitted to hospital Radiography revealed calculus in the left ureter H was again operated upon (catheterisation of the ureter and renal pelvis, followed by *uretero-tomy*), but no calculus was found. Post *operatively he became anuric and serum* N P.N rose to 90 mg% Catheterisation was followed by fall in serum N.P.N and an increase in diuresis. After removal of the catheter he developed pyrexia p to 40 C and had further attack of left renal colic associated with anuria. Catheterisation was once more performed and this again resulted in a fall i serum N P.N to 80 mg% and the temperature dropped to 38 C. He was moved t urological clinic and was once more subjected to catheterisation following which serum

N.P.N. fell from 104 mg% to 27 mg% and the patient's condition improved. Radiography revealed the presence of a calculus in the left renal pelvis which appeared to block the pelvi ureteric junction like a ball-valve. This cal calus together with an additional one were removed from the left renal pelvis by *pyelolithotomy*. Serum calcium was between 9.7 mg% and 9.8 mg%. Post-operatively there was pyrexia but renal function was satisfactory. He developed urinary tract infection which was treated with an acidogenic drug. As this therapy was ineffective he was put on chloromycetin which controlled the urinary tract infection. B.P. was 120/80 mmHg. Serum N.P.N. was 3 mg%.

Three years later (1956) radiography revealed a calculus, the size of a hazel-nut, in the left renal pelvis. Microscopy of the urinary sediment revealed numerous R.B.C. and one or two W.B.C. per high power field. Serum N.P.N. was 3 mg% serum calcium 10.7 mg% and serum phosphorus 4.2 mg%. H had intermittent left renal ache. B.P. was 120/75 mmHg. The calculus was removed from the left renal pelvis by *pyelolithotomy* the operation revealing that the kidney was surrounded by dense fibrous adhesions. Thereafter the patient remained well until 1958 when a further attack of left renal colic occurred. Urography revealed a calculus, the size of a bean, in the upper part of the left ureter with proximal hydronephrosis. There was occasional haematuria. B.P. was 135/75 mmHg. Creatinine clearance was 90 ml per minute, serum N.P.N. 32 mg% serum calcium 10.4 mg% and serum phosphorus 3.1 mg%. The calculus was removed by *pyelolithotomy* the operation revealing the wall of the renal pelvis to be markedly thickened with oedema of the mucous membrane. H was put on a high fluid intake and remained well thereafter. Follow-up examinations after six months and one year respectively revealed satisfactory renal function. Serum N.P.N. was between 32 mg% and 38 mg% serum calcium between 10.6 mg% and 11.9 mg% and serum phosphorus 3.9 mg%.

Urinalysis

1961 Abnormal amounts of cystine, lysine arginine and ornithine (Boström & Hambraeus).

Stone analysis

1950. *Calculus removed from the left renal pelvis that same year* composed of calcium oxalate and ammonium magnesium phosphate (Lehman).

1958 *Calculus removed from the left renal pelvis that same year* cystine stone (roentgen crystallography Lagergren).

Case No. LII 03 08 L.M.L.J. a housewife, born in 1927. Apart from a few episodes of cystitis the patient had not had any other urinary tract symptoms. At the age of 31 she developed duodenal ulceration.

Urinalysis

1961 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Family LIII

Thirty-one individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. LIII 01 03 A.M.C. a house-wife, born in 1908. The patient was married and had one child. She had previous history of diphtheria at the age of 9 (1917), suspected peptic ulceration, with which she was admitted to hospital at the age of 20 (1928), and appendicectomy at the age of 24 (1932).

At the age of 49 (1957) urinary tract symptoms in the form of abdominal pain in the left side commenced. There was no history either of haematuria or dysuria. Six months later (1958) she had a further attack of left sided abdominal pain. Microscopy of the urinary sediment revealed numerous R.B.C. and one or two W.B.C. per high power field and bacteria. Serum N.P.N. was 39 mg. The urographic findings were suggestive of left

ureteric obstruction, but four weeks later urography revealed normal conditions. Thereafter she had attacks of right renal colic off and on. Radiographic studies (1961) revealed a calculus, the size of an almond in the right ureter with marked proximal hydronephrosis. Serum N.P.N was 30 mg% Microscopy of the urinary sediment revealed between ten and fifteen R.B.C. and between two and four W.B.C. per high power field. There was no bacteriuria. The calculus was removed from the right ureter by *ureterolithotomy*

Urinalysis

1961 Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

1961 *Calculus removed from the right ureter that same year* cystine stone (G Hammarsten).

Family LIV

Twelve individuals and generations were examined.

Results. 1 case of homozygous cystinuria. N case of semi-cystinuria.

Case No. LIV 01 03 N E.B., a cook (male), born in 1923 As child the patient had several courses of treatment for pulmonary tuberculosis (1925–1938). When he was 14 years old (1937) urinary tract symptoms in the form of frequency of micturition, dysuria, and occasional haematuria commenced. The following year (1938) he developed pain in the region of the right kidney Microscopy of the urinary sediment revealed between twenty and twenty-five W.B C., between fifteen and twenty R.B C., and a few cystine crystals per high power field. The urine was negative for tubercle bacilli. Radiography did not reveal any bnormalrty on the right side but the left kidney showed calcification of the renal parenchyma and contained a coral stone. *Left nephrectomy* was carried out. The operation specimen was found to be slightly enlarged kidney which showed marked degree of hydronephrosis, only thin rim of renal parenchyma remaining; the renal pelvis contained a large number of calculi and large amount of pus. The possible presence f renal tuberculosis was not verified either bacteriologically or histologically The post-operative course was uneventful. Thereafter the patient was symptom free for eight years. Thereafter he had a further attack of right renal colic (1947). Radiography revealed a calculus in the right ureter from which it was removed by *uretero-lithotomy* Three years later (1950) he was admitted to hospital with cystitis. In 1953 he was once more admitted to hospital with an attack of right renal colic and underwent second *ur terolithotomy* Three years later (1956) an attack of right renal colic recurred and was associated with albuminuria, but urography did not reveal any abnormality Serum N.P.N was 34 mg% B P was 120/80 mmHg. Microscopy of the urinary sediment revealed no or one R.B C one or two W.B.C. and a few hyalin casts per high power field. The urine was negative for tubercle bacilli. The pa tient attended regularly hospital for follow-up examinations. The urine was consistently found to contain albumen but no sugar Serum N.P.N was between 30 and 34 mg% Microscopy of the urinary sediment did not reveal any bnormality In 1957 he underwent appendicectomy Two years later (1959) he ex perienced undue fatigue and abdominal pain off and on. There was radiographic evidence of a calculus, the size of a grain of rice In the right renal pelvis. His urine was still positive for albumen but negative for sugar and tubercle bacilli. B.P was 135/90 mmHg. Two years later radiography revealed that the calculus in the right renal pel is had increased in size. Creatinine clearance was 55 ml per minute. The calculus was removed by *py lolithotomy* The post-operative course was uneventful. The patient was discharged home and instructed to maintain high fluid intake and was put on sodium bicarbonate (1 g four times daily). The following year (1962) he was re-admitted to hospital for investigation of the cause of his progressively increasing fatigue Serum creatinine was then between 3.0 mg% and 3 1 mg% Serum N.P.N varied between 39 mg% and 66 mg% He had a

further attack of right-sided pain but radiography did not reveal any abnormality of the right kidney. Serum uric acid varied between 7.9 mg% and 9.4 mg%.

Urinalysis

1961. Increased concentration of cystine (Trydling).

1961. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1961. *Calculus removed from the right renal pelvis that same year:* cystine stone (Laboratory at the Hospital in Lund).

Family LV

Fifty-two individuals and 4 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. LV 01 03. W. A. O., a house-wife born in 1905. The patient was married and had one child. From the age of *16 (1921)* she had had several episodes of pyelonephritis. *From 1935* to 194 repeated attacks of renal colic occurred. Urography revealed a contracted right kidney. At the age of *41 (1946)* exploratory laparotomy was carried out, the operation revealing cirrhosis of the liver. In 1956 she developed pyelocystitis associated with pyrexia and was put on a course of sulpha drug. The following year (1957) radiography revealed non-function of the right kidney. B.P. was 140/75 mmHg. *Right nephrectomy* was performed, the operation revealing a contracted kidney with perirenal adhesions. Microscopy of the kidney specimen revealed polycystic changes and the histological picture of pyelonephritis. Creatinine clearance was 155 ml per minute. PAH-clearance was 485 ml per minute. The following year (1958) she was operated on for repair of an incisional hernia. Urography did not reveal any abnormality of the left kidney. B.P. was 160/90 mmHg. Thereafter she regularly attended hospital for follow-up of her bacteriuria which was treated with sulpha therapy. She remained well until 1961 when she had an attack of left renal colic associated with pyrexia and anuria. Radiography revealed a calculus in the left ureter. B.P. was 160/80 mmHg. Serum creatinine was 5.5 mg%. Microscopy of the urinary sediment revealed numerous R.B.C. and some W.B.C. per high power field. The ureteric calculus was found to be lodged in the proximal ureter and removed by *pyelo-ureterolithotomy*, a T-tube being left in position for three weeks. On discharge home serum creatinine was 0.9 mg% and she was symptom-free.

Urinalysis

1961. Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Stone analysis

1961. *Calculus removed from the left renal pelvis that same year:* cystine stone (Josephson).

1961. *Calculus removed from the left renal pelvis that same year:* cystine stone (roentgen crystallography, Hambraeus & Lagergren).

Family LVI

Thirty-four individuals and 3 generations were examined.

Results: 5 cases of homozygous cystinuria. 8 cases of semi-cystinuria.

Case No. LVI 05. I. M. O., a house-wife, born in 1911. The patient was married and had four children. Her pregnancies in *1932* and 1949 were complicated by albuminuria. Since childhood she had had repeated episodes of pyelocystitis and after her pregnancy in 1949 she was admitted to hospital with pyelitis. At the age of *43 (1954)* she attended hospital on account of discoloured urine. Microscopy of the urinary sediment revealed one or two R.B.C. per high power field. In 1956 she was admitted to hospital with cerebral haemorrhage associated with pyrexia up to 41.8 C. Microscopy of the urinary sediment revealed a large number of R.B.C. and numerous W.B.C. per high power field. On admission to hospital her B.P. was 170/110 but the patient stated that it had previously been higher. The clinical

picture was thought to be consistent with acute pyelitis and renal hypertension. The patient regularly attended hospital for follow-up of her B.P which was 250/140 mmHg in 1958. She was put on hypotensive drugs and the following year her B.P was 230/130 mmHg. Serum N.P.N was then 24 mg% E.C.G did not reveal any abnormality There was radiographic evidence of changes in the major calices on the left side, a co-existent tumour being suggested in addition. As the radiographic appearances were identical with those in 1956 she was not investigated further

At the age of 51 (1962) she developed left sided abdominal pain associated with pyrexia, a presumptive diagnosis of pyelitis being made. B.P was 200/100 mmHg. The urine was negative for both albumen and sugar Microscopy of the urinary sediment revealed between two and ten R.B.C. and W.B.C. respectively per high power field and a large number of bacteria (rods). Radiography revealed enlargement and hydronephrosis of the left kidney with delayed excretion of opaque medium on this side, and also a *calculus in the left ureter* The right kidney did not show any abnormality The patient was given antibiotics and this therapy controlled the pyelitis. A week after discharge home she was re-admitted to hospital with severe abdominal pain in the right side. B.P was 210/100 mmHg. Microscopy of the urinary sediment revealed numerous bacteria (rods). Serum N.P.N was 56 mg% She had repeated rigors associated with pyrexia exceeding 40 C. Radiography revealed bilateral urinary calculi which had moved distally and were causing obstruction and hydronephrosis on the right side. As the patient was very obese one refrained from subjecting her to an operation. Serum N.P.N rose to 100 mg% but the volume of urine passed per 4 hours was between 1 litre and 2 litres. Following *endoscopic removal* of the ureteric calculus she had again a severe rigor associated with pyrexia exceeding 41 C. The following day she had a further rigor following which she went downhill and died that same day at the age of 52, the cause of death being given as purulent pyelonephritis.

Urinalysis

196.. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Case LVI 09 C. G J B., a male patient, born in 1925 He had no urinary tract symptoms.

Urinalysis

1962. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

N stones available.

Case No LVI 02 02 I. H., a house-wife, born in 1935 The patient was married and had one child. Apart from occasional lumbago she had been healthy until the age of 26 (1961) when she had a transient pyrexia and bacteriuria. Six months later these symptoms recurred. Radiography revealed one large and several small calculi in the right renal pelvis. Her urine was negative for both albumen and sugar Serum N.P.N was 31 mg% *Right nephrectomy* was performed. Operation revealed a normal-sized kidney with a dilated pelvis, containing a coral stone. Histological examination of the operation specimen revealed the picture of chronic pyelonephritis. *The post-operative course was uneventful.* She was put on a high fluid intake round the clock and was given sodium bicarbonate to maintain her urine alkaline.

Urinalysis

1961 Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1961 *Calculus removed from the right renal pelvis that same year* cystine stone (Bjornesjo).

Case No LVI 02 03 K. B. O., an office clerk (female), born in 1941 She had no urinary tract symptoms.

Urinalysis

1962. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

No stones available.

Case No. LVI 02-04 M.O a girl, born in 1949 She had no urinary tract symptoms.

Urinalysis

1962: Abnormal amounts of cystine, lysine arginine, and ornithine (Bostrom & Hambraeus).

Stone analysis

No stones available.

Family LVII

Three individuals and generations were examined.

Results: 1 case of homozygous cystinuria. 1 case of semi-cystinuria.

Case LVII 01 G L.S., a foundry worker born in 1923 The patient was an adopted child and had remained well until the age of 33 (1960) when he developed pain in the region of the bladder The following year (1961) there was retention of urine associated with pain in the urethra. A calculus had got held up in the fossa navicularis urethrae from which it was extracted. Radiography revealed 3 small calculi in the left renal pelvis, bilateral hydronephrosis, particularly on the left side, and the presence of calculus in the bladder The vesical calculus was removed by *suprapubic cystolithotomy* Thereafter the patient was symptom-free.

Urinalysis

1961 Abnormal amounts of cystine lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

1961 *Calculus removed from the urethr that same year* cystine stone (Warén).

1961 *C lculus removed from the bladder that same year* cystine stone, weighing 119.8 g (Warén).

Family LVIII

Nine individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. No case of semi-cystinuria.

Case No. LVIII 01 03 01 M.F W boy born in 1961 When the patient was 8 months old his mother had noticed that he passed small calculi spontaneously for a week, there being no other urinary tract symptoms. Radiography of the urinary tract did not reveal any abnormality

Urinalysis

196.. Abnormal amounts of cystine, lysine, arginine and ornithine (Boström & Hambraeus).

Ston analysis

1962 *Calculus passed spontaneously 1961* cystine stone (oentgen crystallography Lagergren).

Family LIX

Seven individuals and 3 generations were examined.

Results: 1 case of homozygous cystinuria. N case of semi-cystinuria.

Case No. LIX 06 L.P a house-wife, born in Finland in 1926. The patient was married and had one child. At the age of 20 (1946) she experienced frequency of micturition and scalding and passed calculi spontaneously These symptoms recurred at longer or shorter intervals. Her pregnancy at the age of .8 (1954) was complicated by the symptoms of cystitis and albuminuria was found. B P was 160 110 mmHg. Microscopy of the urinary sediment revealed numerous W.B C per high power field. At the follow-up examination six months later the urine was still positiv for albumen. Microscopy of the urinary sediment revealed between ten and fifteen R.B C and numerous W.B.C. per high power field Two years later (1956) she had further episode of cystitis and was put on a course of a sulpha drug. The following year she was admitted to hospital for investigation of the

cause of recurrent cystitis. B.P. was 195/120 mmHg. Radiography revealed a large calculus in the right renal pelvis and also right hydronephrosis, and ureteric obstruction. The patient refused to be operated upon and took her own discharge. Thereafter she did not seek medical advice despite dysuria, frequency of micturition, and macroscopic haematuria which lasted a few days (1959). In 1962 her doctor prevailed upon her to go to hospital. On admission B.P. was 210/120 mmHg. Her urine did not contain either albumen or sugar. Microscopy of the urinary sediment revealed between three and six W.B.C. per high power field. A few days after admission numerous bacteria and an increase in the number of W.B.C. per high power field were noted in addition. Serum creatinine was 0.7 mg%. Radiography revealed a coral stone in the right renal pelvis with associated hydronephrosis; the left kidney did not show any abnormality. Treatment with antibiotics resulted in controlling the urinary tract infection. The coral stone was removed by *pyelolithotomy*. The post-operative course was uneventful. The patient was discharged home and put on a course of sulpha drug.

Eight days after discharge home she passed calculi spontaneously. B.P. was then 170/100 mmHg. Thereafter she remained well. Serum creatinine was 0.7 mg% and creatinine clearance 57 ml per minute. Radiography revealed right hydronephrosis and the presence of several calculi in the right kidney: the left kidney still showed no abnormality. The patient was put on a high fluid intake and this combined with isosthenuria (specific gravity up to 1.017) resulted in a 24-hour output of urine of 5 litres.

Urinalysis

1962. Abnormal amounts of cystine, lysine, arginine, and ornithine (Boström & Hambraeus).

Stone analysis

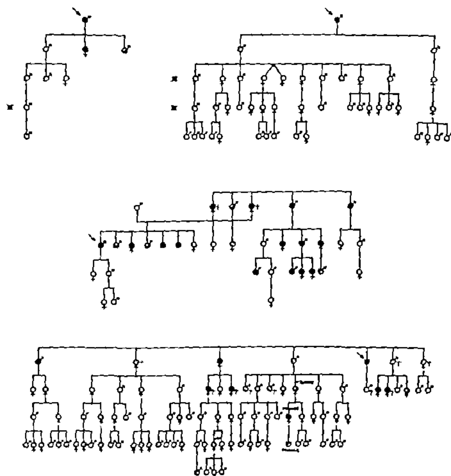
1962. *Calculi passed spontaneously: cystine stones (Laboratory at the Hospital in Kiruna).*

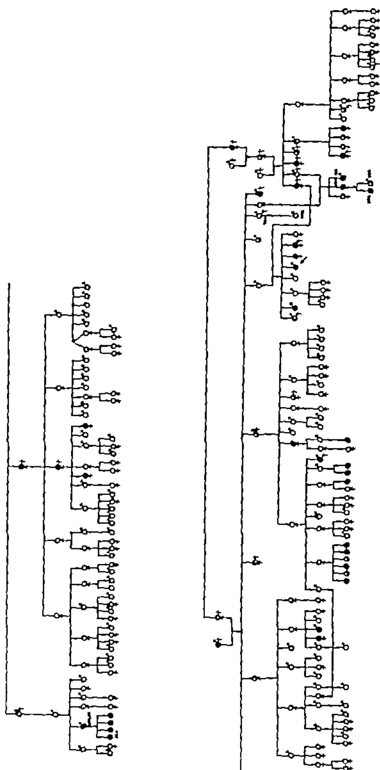
DATA ON RELATIVES OF THE ABOVE CYSTINURIC

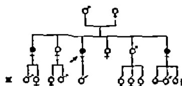
The patient (LIX 06) was the sixth of eight children. One of her brothers had a history of a single attack of renal colic culminating in the spontaneous passage of a calculus. One of her sisters had suffered from renal failure but had not formed stones. She died in child-birth. With the exception of one sister all her siblings were resident in Finland. Only those members of her family were examined who were resident in Sweden.

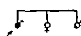
Symbols Used In the Family Trees

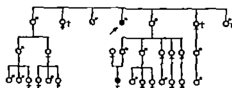
♂ – Male
♀ – Female
○ – Sex unknown
● ♀ – Homozygous cystinuric
♂ ♀ – Healthy individual
♂ ♀ ● – Not examined

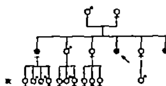


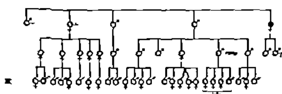


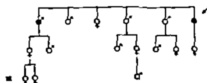


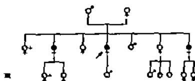


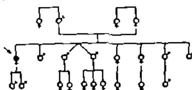


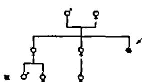


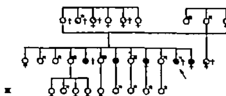


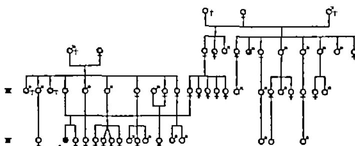


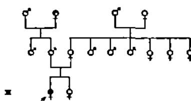


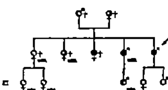


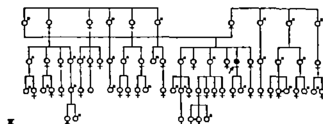


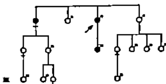


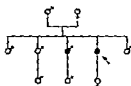


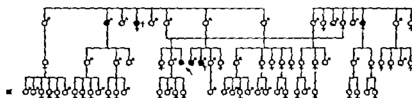


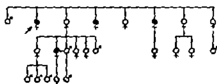


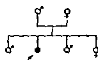


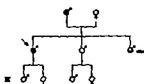


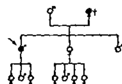


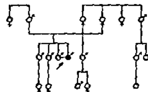


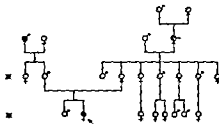


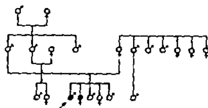


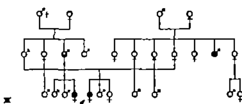


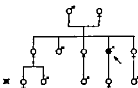


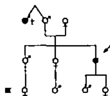


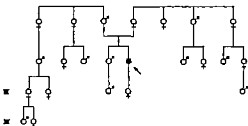


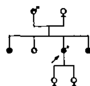




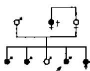


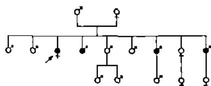


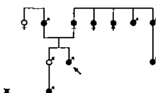


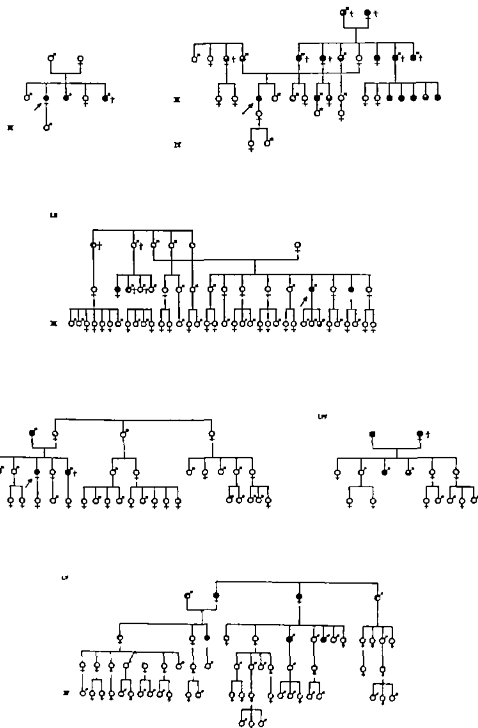


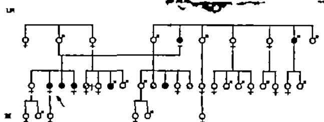


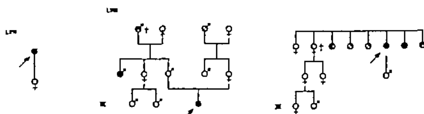












ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 412

ARTICLES DEDICATED

TO

Bertel von Bonsdorff

ON HIS

60th Birthday

by

his Former and Present Pupils

ACCOMPANIES VOL. 175

HELSINGFORS 1964

TO

BERTEL von BONSDORFF

ON HIS

60th BIRTHDAY

ACTA MEDICA SCANDINAVICA

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Bertel von Bonsdorff

Henrik Tikkanen del.



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Professor of Internal Medicine and Head of the Fourth Department of Medicine University of Helsingfors.

Bertel von Bonsdorff

Curriculum vitae

Born: April 19 1904

1921 Matriculation examination, Nya Svenska Läroverket, Helsingfors

1924 Med. Cand., University of Helsingfors

1931 Med. Lic.

1933 M.D. (dissertation)

1932–1934 Resident, Department of Internal Medicine, Maria Hospital, Helsingfors

1934–1935 Resident, I Department of Medicine, University of Helsingfors

1935–1938 Assistant Teacher I Department of Medicine University of Helsingfors

1935–1947 Docent in Internal Medicine University of Helsingfors

1939–1941 Assistant Chief Physician, II Department of Medicine, University of Helsingfors

1941–1946 Assistant Chief Physician, I Department of Medicine, University of Helsingfors

1946 Acting Professor of Internal Medicine, University of Helsingfors

1947– Professor of Internal Medicine (Swedish tuition language) and Head of the IV Department of Internal Medicine, University of Helsingfors

Studies Abroad

1931–1932 Germany (Medizinische Klinik, University of Göttingen)

1935–1936 Scandinavia (various university hospitals)

1938 U.S.A. (Research Fellow at the Thorndike Memorial Laboratory Boston City Hospital Boston, Mass.)

Military Service

1929 Resident at the Military Hospital, Viborg

1939–1944 During the wars physician in charge of various military hospitals

1943 Major Army Medical Corps

Inter-Scandinavian Academic Activities

Expert called upon to give advice on the merits of the candidates for the chair of Internal Medicine University of Lund, Sweden, 1949 (twice) Oslo, Norway 1955 (twice) 1962

President of the 26th Scandinavian Congress of Internal Medicine, Helsingfors, 1958 of the 4th Scandinavian Congress of Nephrology Helsingfors, 1959

Scientific Societies

President, the Finnish Society for Internal Medicine (1948–1950) Finska Läkaresällskapet (Societas Medicorum Fennica) (1953, 1954) Finska Vetenskapssocieteten (Societas Scientiarum Fennica) (1962–1963)

Member Societas pro Fauna et Flora Fennica (1936) Finska Vetenskapssocieteten (1939)

Representative of Finland, the Board of the International Association of Internal Medicine (1958–)

Vice President, Société européenne d'hématologie (1951–)

Foreign member Svenska Läkaresällskapet (1949 Schweizerische Gesellschaft für Hämatologie (1951), Dansk Selskab for intern Medicin (1953), Det norske medicinske Selskab (1954) Kungliga Vetenskaps-Societeten, Uppsala (1957)

Honorary member Medicinarklubben Thorax (Society of the Swedish-speaking medical students) (1954) *Sjuksköterskeföreningen i Finland* (The Finnish Nurses Association) (1955 Finska Läkaresällskapet (1960), Nylands nation (Nyland Student Fraternity) (1963)

Editorial Activities

Chief Editor Finska Läkaresällskapets Handlingar

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President, Samfundet Folkhälsan i Svenska Finland (1962—)

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President, Finlandssvenska Samfundet (Society of the Swedish-speaking population) (1949—1952)

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Member of the boards of Oy Medica Ab and Oy Hesperus Ab (1946—1947)

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E. F. Rosenqvist prize (Societas Medicorum Fennica) 1938

Helena Lundqvist prize (Societas Medicorum Fennica) 1953

Orders and Medals

Commander of the Lion of Finland, Cross of Liberty 3rd class with Red Cross, Cross of Liberty 4th class for War-time Merits, Memorial Medal of the War 1939—1940, Memorial Medal of the War 1941—1945, Medal for Meritorions Deeds of Svenska Finlands Folkting (Folk-thing of Swedish Finland) Long Service Medal (30 years) of Central Chamber of Commerce in Finland, Badge for Merits of Samfundet Folkhälsan i Svenska Finland, Knight of St. John.

Publications

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2. von Bonsdorff, B. & Räihä, C. E.: *Die pulsatorischen Verschiebungen der Cornea beim Kaninchen.* Skand. Arch. Physiol. *48*: 234, 1926.
3. Granit, R. & von Bonsdorff, B.: *Zur Kenntnis der humoral übertragbaren Herzvagusreizwirkung. I–II.* Skand. Arch. Physiol. *51*: 249 & 305, 1927.
4. von Bonsdorff, B. & Granit, R.: *Über eine Steigerung des Reizeffektes am Herzvagus der Ratte bei Mangel an fettlöslichen Vitaminen in der Nahrung.* Skand. Arch. Physiol. *55*: 30, 1929, also in Finska Läk. Sällsk. Handl. *70*: 79, 1928.
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Editorial Activities

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73. von Bonsdorff, B.: *L'anémie pernicieuse bothriocéphalique.* Sem. Hôp. Paris (Médecine dans le monde) *32* No. 1 1956.

74. von Bonsdorff, B.. *Diphyllobothrium latum as a cause of pernicious anemia.* Exp. Parasit. *5* 207 1956.

75. von Bonsdorff, B.. *Cardiology in Finland.* Nord. Med. *56.* 1257 1956.

76. von Bonsdorff, B.: *Pathogenesis of vitamin B_{12} deficiency, with special reference to tapeworm pernicious anemia,* in "Vitamin B_{12} and Intrinsic factor" (Heinrich H. C., ed.) Enke, Stuttgart, 1957 p. 311

77. von Bonsdorff, B.. *Vitamin B_{12} deficiency in carriers of Diphyllobothrium latum,* in "Proc. 6th International Congr. Tropical Medicine and Malaria, Lisbon, 1958" Vol. 2, Porto, 1958, p. 541

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79. von Bonsdorff, B.: *Bred bandmask som orsak till B_{12}-vitaminbrist.* Farm. notisblad *68* 183, 1959.

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81. von Bonsdorff, B. *Robert Ehrström. Minnestal hållet vid Finska Vetenskaps-societetens sammanträde den 21 mars 1960* Soc. Scient. Fenn. Årsbok *38* C. Fasc. 2, 1960.

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88. von Bonsdorff, B.: *Robert Tigerstedt*, in "Finländska Gestalter I" (Mörtelin, O., ed.) Ekenäs Tryckeri, Ekenäs, 1961 p. 149

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92. von Bonsdorff, B., Gräsbeck, R., Nyberg, W. & Saarni, M.: *Statistische Verteilung der Vitamin-B_{12}-Werte im Blutserum in einer mit breitem Bandwurm (Diphyllobothrium latum) stark infizierten Bevölkerungsgruppe sowie Abhängigkeit der hämatologischen Befunde vom Vitamin-B_{12}-Spiegel*, in "Vitamin B_{12} und Intrinsic factor" (Heinrich, H. C., ed.) Enke, Stuttgart, 1962, p. 607

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Editorial

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The main landmarks in the career of Bertel von Bonsdorff are apparent from his *curriculum vitae* and his scientific work from the list of his publications and the greetings from his former chief and predecessor, the octagenarian Fredrik Saltzman. To these data his pupils wish to add some remarks of their own.

The achievements of individuals should be judged against their historical background. Those citizens of Finland who today have reached the age of 60 have experienced remarkable historical events. In 1904, when Bertel von Bonsdorff was born, Finland was still part of Russia, subject to intense political persecution aiming at russification and steadily losing what little remained of her guaranteed political autonomy. In 1914 the First World War started and in 1917, amidst the chaos of the Russian revolution, Finland declared herself independent. Then there was an extremely bloody war in which the white government conquered the reds and ousted the Russians. As seems to be the rule in all young countries, the early years of Finnish independence were characterized by political instability. Noteworthy in the present connexion was the development of strong tensions between the Finnish-speaking majority of the population and the Swedish-speaking minority. The resentment against the Swedish language is historically attributable to its earlier dominant position in Finland as a heritage from the 700-year-long Swedish rule, which ended in 1809. In 1939—1940 there was the Soviet Finnish war — the famous Winter War — which ended in the annexion of some of the eastern parts of the country by the USSR. And 1941—1944 there was a new war against the Soviet Union, ending in the present geographic and neutral status of the country.

That it was possible to perform scientific investigations and to achieve an internationally recognized medical competence in spite of this turmoil is remarkable. However, von Bonsdorff managed to do this. For instance, he used the time he was in the army extremely well, in contrast to other people who tended to spend their military leisure hours (and they are many during a long war!) doing nothing. As a young recruit he made observations leading to a method for detecting latent tetany (5, 85), and during the Second World War while in charge of an army hospital, he made numerous experiments on tapeworm anaemia (no doubt readily assisted by the soldiers, who found the swallowing of gastric tubes more pleasant than going back to the front). Also, he used this time to write his textbooks for nurses (M1, M2), which

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incidentally are very useful primers for medical students and which deserve to be translated into some world language.

In spite of these achievements the wars of course, greatly interfered with his scientific work. The Winter War in 1939 interrupted the very promising studies on serum proteins that he was making with Waldenström in Sweden. He had only time to write a few acute observations concerning crystallizing paraproteins (31) and the use of the ultra centrifuge in medicine (30)

Soon after the end of the war Bertel von Bonsdorff (or rather "Bebe" because that is what we call him) was appointed Professor of Internal Medicine at the University of Helsingfors and installed as Head of the IV Department of Medicine.

Von Bonsdorff's department is very small However we feel that the impact of his department is out of proportion to its small size. This is shown by the present positions of the invited authors of these articles, and — we hope — also by the contents of their publications.

The explanation for this development is that von Bonsdorff is a born leader and inspirer of scientific research. He has a keen apprehension of which medical fields need to be developed and who are the persons suitable for doing the job. He picks out potential scientists, injects them with the highly contagious research virus, and after a suitable incubation time, produces mature scientists.

Suffice t to mention one example: Seeing that nephrology was grossly underdeveloped in this country he got one of his pupils interested in this subject. Suddenly when the need for an artificial kidney became pressing the renal ward simply had to be attached to his department. Thus in 1960 his staff doubled in that one nephrologist, two additional residents and one new intern were attached to the department.

In von Bonsdorff's department there is virtually no space for performing experimental research and the state-owned University has given its clinical departments neither personnel nor space for research purposes. Bebe suddenly crystallized the idea. Let the scientists pool their grants, rent an apartment and found a private research institute. This was the beginning of the Minerva Foundation Institute for Medical Research. This institute has turned out to be a very successful enterprise and has introduced an almost unprecedented kind of scientific institution into this country Recently the Samfundet Folkhälsan Svenska Finland (The Society for Public Health in Swedish-speaking Finland) the president of which is von Bonsdorff, founded an Institute for Genetics, which was attached to the Minerva Institute. Another private institution inspired by v Bonsdorff is the Parasitological Institute of the Finnish Scientific Society attached to Åbo Academy

Scientists are willing to forego much material advantage to be able to do research, but there is a minimum living standard which only the highly eccentric will sacrifice, whereas others in such a situation search for more profitable practical assignments. Realizing this and the great costs involved in medical research, Bebe has done his best to acquire sufficient funds for the researchers. For this we are all grateful.

Bertel von Bonsdorff is Swedish-language Professor of Internal Medicine. For those who are acquainted with linguistic minority problems in their own countries, this fact may strike a chord. It is not easy to belong to a minority population, and few problems stir emotions more readily Just look at today's newspaper!

Since the burial of extreme nationalism in Europe under the ruins of the Second World War the people of Finland have definitely sobered up on the language problem, and at the present moment this is better handled than in many other countries, though there is still a long way to go The young academic generation especially has shown encouraging signs of tolerance and a democratic spirit. Some serious language problems still remain, such as that those who aspire to high academic positions, in most instances have to pass exceedingly exacting examinations to prove that they have "complete command of the lecturing language. Analogous requirements in the United States and Sweden would sweep out a large part of their academic staffs

In the nineteen-thirties the situation was altogether different. A wave of Finnish nationalism swept the country and threatened to eradicate all tuition in the Swedish language. Bertel von Bonsdorff belongs to the generation which had to ride these storms. His attitude has been characterized by sound judgement and tolerance coupled with strict legality He is nowadays generally accepted as one of the leading medical men of our country The important role he plays in Inter-Scandinavian medical cooperation also deserves special mention

Speaking of language, t will be noted that Bertel von Bonsdorff has an extremely fluent pen. In his youth he took an active part in student journalism. His recent historical 250-page-long account "Finska Läkaresällskapet 125 år historik 1935—1960" (M3) is extremely well written His biography "Robert Tigerstedt" (88) is a pearl and in "Robert Ehrström (81 he excels in writing between the lines. "En läkartidnings uppgift" (The mission of a medical journal) (66) written in honour of the Svenska Läkartidningen, deserves to be read by all those who contribute to medical literature.

There are many kinds of professors. Though less frequent than in some countries, the Geheimrat nd Big Boss are not uncommon here. It is indeed remarkable nd

perhaps worth of closer attention by psychologists, how soon some personalities change after appointment to professorships. Bebe is remarkably free from "professorism" and he uses wit — occasionally spiced with irony — but not bad temper to rule his department and his numerous pupils. We do not regard him as a boss but as our sincere friend and senior collaborator

Happy birthday!

On behalf of your pupils

Ralph Gräsbeck *Bror Axel Lamberg* *Otto Wegelius*

Bertel von Bonsdorff

Greetings from his Old Teacher and Predecessor

Dear friend

Your pupils and collaborators have asked me to write a few introductory words to this volume published in your honour. I feel it a great privilege to have this opportunity to congratulate you on your 60th birthday. It seldom happens that a former teacher is able to salute and felicitate his pupil when the latter has reached the meridian of life, after years filled with work and success.

Our acquaintance dates back over more than 40 years, and I have followed your career from the time you were a university student until today, when you occupy a leading position in the medical profession in the Scandinavian countries. During these years my thoughts have often been engaged with questions relating to learn

Grüsse von seinem alten Lehrer und Vorgänger

Lieber Freund,

Deine Schüler haben mich gebeten, diese Festschrift mit einigen Worten einzuleiten. Für die Gelegenheit meine Glückwünsche anlässlich Deines 60. Geburtstages in dieser Weise aussprechen zu können, bin ich sehr dankbar. Es kommt nicht oft vor, dass ein alter Lehrer seinen Schüler grüssen und beglückwünschen kann, wenn dieser nach Jahren voller Arbeit und Erfolge schon die Mittagshöhe des Lebens erreicht hat.

Unsere Bekanntschaft ist über 40 Jahre alt und ich habe deine Laufbahn von Deinen Studentenjahren bis in die Gegenwart verfolgt, wo Du jetzt eine führende Stelle in der medizinischen Welt hier in den Nordischen Ländern einnimmst.

Im Lauf dieser Jahre haben sich die Gedanken oft beschäftigt mit Fragen über das

ing and tuition, to research, medical training, the activities, concerns and joys of a university teacher and to the role of investigation and the investigator in these connexions. A thorough and comprehensive training is a necessary basis for successful teaching. But a good teacher must be an investigator himself, and above all he must be able to inspire and lead his pupils to undertake research. In most cases the work of an individual is but a small contribution, yet what he has begun can be developed by collaborators, pupils and later generations; it can thrive and bear fruit in the soil the teacher has helped to create. Without the perspective and the fighting spirit connected with research, the activity of a teacher becomes shallow and wanes.

It appears to me that in many different respects your course in life forms a striking illustration of what I have just said. By preliminary studies and investigations in physiology and biochemistry you laid a good foundation for your later work. When you were only a medical student, you published a mature paper on tetany and the demonstration of a tetanic diathesis, or latent tetany, by a combined tourniquet and ventilation test, and you were one of the early workers in the modern field of blood protein research.

Your doctoral thesis "Zur Methodik der Blutdruckmessung" (10) dealing with a subject on the border between physiology and internal medicine, revealed your capacity to explore a vast problem with thoroughness and scientific imagination, to evaluate the results critically and to present them in the clear and moderate form so often encountered in your writing. Later you have repeatedly and in a num-

Lernen, Lehren und Forschen, über die medizinische Ausbildung, über das Wirken des akademischen Lehrers und über dessen Kummer und Freude, sowie über die Bedeutung der Forschung und des Forschens in diesem Zusammenhang.

Gediegene und vielseitige Ausbildung ist eine Voraussetzung für ein erfolgreiches Wirken als Lehrer. Aber ein guter akademischer Lehrer muss auch selbst Forscher sein. Vor allem muss er die Fähigkeit besitzen, seine Schüler zum Forschen zu inspirieren und ihnen bei Forschungsaufgaben durch sein Wissen und seine Ratschläge zu helfen. Wissenschaftliches Forschen eines Einzelnen ist meistens Stückwerk, aber durch Mitarbeiter, Schüler und spätere Nachfolger kann das Werk weitergeführt werden und einst in dem mit Hilfe des Lehrers geschaffenen Boden gedeihen und Früchte tragen. Ohne die Tiefe und den Geist des Forschens verflacht sich die Arbeit des Lehrers und siecht schliesslich dahin.

Dein Lebensgang scheint mir das Obenerwähnte in vorbildlicher Weise und vielseitiger Art zu veranschaulichen. Durch Studien und Untersuchungen im Rahmen der Physiologie und der medizinischen Chemie hast Du einen guten Grund für Deine spätere Tätigkeit geschaffen. Schon als cand.med. hast Du eine reife Arbeit veröffentlicht über Tetanie und den Nachweis der Tetaniebereitschaft oder der latenten Tetanie durch eine kombinierte Tourniquet-Ventilationsprobe. An der neuzeitlichen Blutproteinforschung hast Du Dich früh beteiligt.

In Deiner Habilitationsschrift "Zur Methodik der Blutdruckmessung" (10) zeigst Du die Fähigkeit, Dich gründlich und mit wissenschaftlicher Phantasie in ein grosses Problem zu vertiefen, die Ergebnisse kritisch zu beurteilen und diese in jener massvollen Art, der wir in Deinen Schriften so häufig begegnen, klar

ber of different connexions concerned yourself with questions of cardiology. I will only recall your paper on "Myocardial disease of obscure origin" (36). It is useful to trace the boundaries of our knowledge and skill on the basis of a carefully examined series, and to point out the need of continued research.

Gradually you became more and more interested in haematological problems. This field encompasses the most extensive and most successful of your studies.

Several generations of Finnish investigators had already struggled with the problem of pernicious anaemia and the tapeworm *(Diphyllobothrium latum)* when it was finally and convincingly solved by you. It is impossible here to list all the methodological experiments and interesting observations which led you to your goal, or your contributions to the biology of the tapeworm. Suffice it to mention the result: In the human intestine the tapeworm consumes large amounts of vitamin B_1, resulting in a deficiency state, which manifests itself in the form of pernicious anaemia. The old hypothesis that the anaemia is due to some poison perhaps produced by the tapeworm, had long been doubted, and today it is generally accepted that pernicious anaemia is a deficiency disease. The anaemia caused by the tapeworm can be regarded as a true vitamin B deficiency disease in which the causative factor is known. In the so-called genuine pernicious anaemia the pathophysiological mechanism has been clarified in part, but is not yet understood in all its details.

In what I have said above I have only outlined some of the main features of your scientific achievements. Your research

darzustellen. Später hast Du Dich wiederholt mit kardiologischen Fragen beschäftigt. Ich will nur an Deine Arbeit über "Myocardial disease of obscure origin" (36) erinnern. Es ist nützlich, an einem gut untersuchten Material die Grenzen unseres Wissens und Könnens zu zeigen und auf die Notwendigkeit fortgesetzter Forschung hinzuweisen.

Allmählich haben aber hämatologische Fragen Dein Interesse immer mehr gefesselt. Der grösste und erfolgreichste Teil deiner Forschung bewegt sich auf diesem Gebiet.

Nachdem schon viele Generationen finnländischer Forscher mit dem Problem "Die perniziöse Anämie und der breite Bandwurm (Diphyllobothrium latum)" gerungen hatten, ist es Dir gelungen, es in überzeugender Weise zu lösen. Es ist nicht möglich hier alle methodischen Experimente und alle die interessanten Beobachtungen zu erwähnen, die zum Ziele führten. Dasselbe gilt für Deine Beiträge zur Kenntnis der Biologie des Bandwurms. Es genügt, auf das Endergebnis hinzuweisen. Der Bandwurm verspeist im Darm des Menschen grosse Mengen Vitamin B_1 und ruft dadurch einen Mangel hervor, der zur perniziösen Anämie führt. Die alte Hypothese, die Anämie sei durch ein Gift hervorgerufen, ist schon lange bezweifelt worden. Nunmehr wird die perniziöse Anämie als eine Mangelkrankheit aufgefasst. Die vom Bandwurm bedingte Anämie muss, wie von finnländischer Seite schon früher behauptet worden ist, als eine echte Form der perniziösen Anämie mit einem jetzt in Einzelheiten bekannten auslösenden Faktor betrachtet werden können — neben der sogenannten kryptogenetischen perniziösen Anämie, wo der B-Vitamin-Mangel nachgewiesen ist, dessen Entstehen aber noch nicht in allen Einzelheiten klargelegt werden konnte.

Hier habe ich nur die Hauptzüge Deines wissenschaftlichen Schaffens andeuten können.

work has been closely allied with your activity as a university teacher. The way in which you have stimulated and guided numerous talented pupils to pursue investigations of a high quality is reflected in papers from the IV Medical University Department in Helsingfors. This volume shows the level and intensity of the scientific work performed by your former and present pupils. In publishing it, your collaborators and pupils have endeavoured to express their appreciation and their gratitude. They are grateful not only for what you have given them on a scientific and academic level. They also wish to thank you for the human aspect of your relations with them. There has never been any doubt about the easy and friendly atmosphere prevailing in your department. In connexion with your activity as a teacher mention should also be made of two excellent textbooks for nurses, which have run into many editions: "General Pathology" (M1) and "Internal Medicine" (M2) which are also useful reading for members of the medical profession.

Besides being active as a research worker and teacher you have made important contributions in the field of social medicine and preventive health, and worked for the benefit of various medical Institutions in Finland — *the Finska Läkaresällskapet*, the Samfundet Folkhälsan, the Sigrid Jusélius Foundation, the Minerva Foundation Institute for Medical Research and Konkordia Hospital. Outside Finland, too, you have been an appreciated collaborator in the Scandinavian Association for Internal Medicine, the Board of Editors of the journal "Nordisk Medicin", the Board of the

Daran schliesst sich Dein Wirken als akademischer Lehrer. Die Art, in welcher Du zahlreiche begabte Schüler zu hochklassigen Forschungsleistungen geleitet und stimuliert hast, geht aus den Schriften aus der I medizinischen Universitätsklinik in Helsingfors hervor. Auch die hier vorliegende Festschrift zeigt das Niveau und die Intensität der Forschung deiner ehemaligen und heutigen Schüler. Deine Schüler und Mitarbeiter bezeigen Dir durch diese Schrift ihre Hochschätzung und ihre Dankbarkeit. Diese Dankbarkeit gilt aber nicht nur Deinen fachlichen Leistungen. Sie bezieht sich auch auf das, was sie in verschiedener Weise rein menschlich von Dir empfangen haben. Man kann den schlichten, kameradschaftlichen Ton nicht überhören, der in Deiner Klinik herrscht. Eng mit Deiner Lehrtätigkeit verknüpft sind Deine beiden vorzüglichen, in mehreren Ausgaben erschienenen und auch von Medizinern geschätzten Lehrbücher für Krankenschwestern: „Allmän sjukdomslära" (Allgemeine Krankheitslehre) (M1) und „Inre medicin" (Innere Medizin) (M2).

Schliesslich möchte ich hinweisen auf Deine aktive Beteiligung an sozialmedizinischen Unternehmungen sowie an der vorbeugenden Gesundheitspflege wie auch auf Deine Arbeit für verschiedene medizinische Institutionen in Finnland wie für Die Finnländische Ärztegesellschaft, Samfundet Folkhälsan, Sigrid Jusélius Stiftung, Das medizinische Forschungsinstitut Minerva und das Krankenhaus Konkordia. Auch im Auslande bist Du hochgeschätzt, so zum Beispiel in der Nordischen Gesellschaft für innere Medizin, in der Redaktion der Zeitschrift Nordisk Medicin, im Vorstand des Nordisk Insulinfond in Dänemark und in der Internationalen Gesellschaft für innere Medizin.

Diese Aufzählung ist bei weitem nicht vollständig. Sie könnte unter anderem durch Erwähnung vieler Aufträge im öffentlichen und

Nordisk Insulinfond in Denmark and in the International Association for Internal Medicine. This list is far from complete. I could add, for instance, various tasks with which you have been entrusted in the sphere of civic and economic life in this country. This shows the wide scope of your life's achievement, your readiness to accept tasks involving responsibility and your alacrity in coping with a heavy burden of work. And all this without making any show of it, without paying any tribute to the advertising spirit of our day.

May many years of activity still be granted to you, for the benefit of science, and for the benefit and joy of your friends.

wirtschaftlichen Leben ergänzt werden.

Alles bezeugt die Vielseitigkeit Deines Lebenswerkes, die Bereitwilligkeit verantwortungsvolle Aufträge zu übernehmen, die Fähigkeit grosse und schwierige Arbeiten rasch zu erledigen, und das alles ohne die Propagandatrommel einer reklamehungrigen Zeit zu rühren.

Möge es Dir vergönnt sein, noch durch viele Jahre Deine Arbeit fortzusetzen zum Frommen der Wissenschaft wie zur Hilfe und Freude Deiner Freunde.

Fredrik Saltzman

Acta Medica Scandinavica. Suppl. 412, 1964

From the Folkhälsan Institute for Genetics, the Minerva Foundation Institute for Medical Research, the Fourth Department of Medicine of the University of Helsingfors, the Second Department of Medicine of the Deaconess Hospital, and the Second Department of Pathology of the University of Helsinki, Helsingfors, Finland.

XX Sex Chromosomes in a Human Male

First Case

By

ALBERT DE LA CHAPELLE, HERMAN HORTLING, MIKKO NIEMI AND JOHAN WENNSTRÖM

To the best of our knowledge, no case has so far been published of a human male with a pure XX sex chromosome constitution. The karyotype 46/XX has been found in several instances of true hermaphroditism, as originally reported (17, 20). In mosaicism, a 46/XX stemline is not infrequently associated with other stemlines, such as 47/XXY (5, 11, 18, 27).

The purpose of this report is to present evidence of the existence of an XX human male. The study involves an extensive cytogenetical and blood group investigation of the propositus and his family as well as clinical data on the propositus. Histochemical investigations are reported on the tissue cultures established from testicular material of the propositus.

Case Report

The propositus, a male aged 19, was referred to an endocrinologist on account of hypogonadism. At his birth, which had been uneventful, he weighed 3200 g; the maternal age was 28 and paternal age 26 years. H. developed normally as a child, and except for minor infections, had always been healthy. There had been nocturnal enuresis until the age of 13.

H. went through elementary school and was thereafter employed as a manual labourer. He was recognized as a pupil of low-normal intelligence at school.

At 14 years of age, some signs of puberty became apparent. His voice grew deeper, there was growth of axillary and pubic hair and the penis began to grow. There had been no growth of beard, however, and he had never shaved before he received the first course of hormone treatment. His hypogonadism had not been noticed by anyone, not even by his parents, since his general appearance was that of a normal male. The libido was normal. H. had experienced no sexual intercourse and erections of the penis had occurred only rarely. H. had never observed any pollutions. Because of the smallness of the penis and testes he consulted a general practitioner at the age of 19, and was referred to one of us for further examination.

He was 172 cm tall and weighed 72 kg. The arm-span was 174 cm and the pubis-to-sole distance 91.5 cm. H.'s general appearance was that of an athletic, slightly obese, otherwise normal-looking young male (Figs. 1–2). There was no clear-cut gynaecomastia, but the areolae were somewhat larger than normal and the mammary

region contained more fat than in normal males. Glandular tissue could not be palpated. There was no growth of beard but axillary and pubic hair were of normal abundance and distribution.

The penis was small, 4 cm long but of almost normal thickness (Fig. 3). The preputium could be retracted over normal glans with normally situated external urethral meatus. The scrotum was smaller than normal and contained two descended testes. The left one was 2 ½ cm long and of soft consistency. The right one was very small, 1 cm, about the size of a pea, and soft. Epididymal tissue and a funicle were felt on both sides. On rectal examination the prostate was found to be normal.



Fig. 3. The genitals of the patient. The scrotum contains two small testes, of which only the larger left one can be seen in this figure.

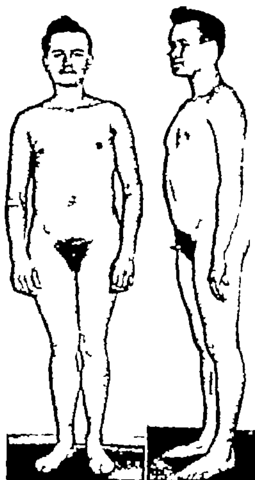


Fig. 1—2. The patient's normal general appearance.

General physical examination revealed nothing of particular interest. Thyroid and adrenal functions were normal. The function of the lungs, heart, liver and kidneys was normal, too. X-ray pictures of the skull and the heart and lungs showed nothing noteworthy. Urography showed normal-looking kidneys, renal pelves, and ureters.

Radiographs of the hands and wrists (16) revealed a bone age of 17 ½ years. The skeletal maturation was thus slightly delayed. The 24-hour urinary gonadotrophin excretion was over 40 mouse units.

The patient was discharged from the ward and given a course of methyltestosterone treatment (25 mg sublingually once a day for three months). This caused growth of some beard, the appearance of acne in the faces, and general masculinization. About two months after the end of the course of hormone treatment, the following values for the 24-hour excretion of hormones in the urine were obtained: gonadotrophins < 10 mouse units, 17-ketosteroids 9.8 and 6.5 mg, 17-hydroxycorticosteroids 15.8 and 16.1 mg, oestrone 0.9 and 0.4 μg, and oestriol 3.9 and 2.2 μg. No oestradiol could be detected in two urine samples.

Half a year after the first admission, laparotomy was performed. Through a transverse suprapubic incision, the intra-abdominal pelvic region was visualized and thoroughly palpated. The whole



F g 4 a—d Biopsy pecimen f the left testis ( ) The histol gy is dominated by interstitial tissue 40 (b) I th lower half f the pictur an adenomatous nodul f Leyd g cells an be seen A hy l ne acell lar membrane around the immatur tubul 90 ( ) Among th Ley d g ll mall fibrocyt -lik interstitial ell ar seen
tain Se tol ll only the Leyd g ll f the m

region was typically male. The spermatic cords were palpated on both sides. There was nothing t suggest the presence of even rudimentary female internal sex organs such as uterus, Fallopian tubes, or ovaries. The appendix ppeared normal. The adrenals, kidneys, spleen and liver were of normal size and consistency upon palpation. However the gall-bladder contained an abundance of small concrements. Because of this finding, the suprapubic incision was closed after prophylactic appendectomy and an upper paramedian incision was made and cholecystectomy performed in the usual way during the same seance. Peroperative cholangiography revealed choledochus of normal width, free of stones or strictures. The stomach appeared normal.

Recovery from the operations was uneventful and the patient was discharged from the hospital in good condition on the ninth postoperativ day

Testicular Histology

Operative testicular biopsies were made on both sides. The skin and tunica albuginea were incised and some testicular tissue was squeezed out by gentle pressure on the testis. Specimens of suitable size were removed with a scalpel and divided into two parts, one for tissue cultures, and the other for histological examination. The testicular tissue appeared normal macroscopically

The biopsy specimens were serially sectioned and stained with H and E and with van Gieson s picrofuchsin.

The histological picture was much alike in both testes. It was clearly dominated by an overgrowth of nterstitial tissue (Fig 4*a*) the seminiferous tubules being small and few in number The mean tubular diameter vas 95 μm, with a range from 65 to 130 μm. The relative area of the two testicular tissue components was estimated by projecting a cross-section of both biopsy specimens on a piece of paper drawing the tubules and cutting out the area covered by these. By weighing the pieces of paper the tubules were found to constitute only 14 per cent of the total area of the testis, while the rest was occupied by the interstitial Leydig cells.

The Leydig cells were mostly of a well-developed normal appearance (Fig 4*d*) but sometimes small, fibrocyte-like interstitial cells could also be observed (Figs. 4*a* and *c*) Some of the Leydig cells were enlarged showing more than the usual eosinophilia in their cytoplasm, occasional hyaline bodies were also seen. The interstitial cells were mostly scattered in the interstices, but cell cords were sometimes seen to form large cell clumps or nodules (Fig 4*b*)

The seminiferous tubules mostly lacked a lumen They were often surrounded by an acellular collagen membrane (Fig 4*b*) and slightly increased peritubular fibrosis was also noticeable The germinal epithelium was low and usually contained very few cells. These were differentiated but not fully mature Sertoli cells. In a few tubules immature prepubertal epithelium was seen with a few spermatogonia but spermatogenesis was not seen in any of them.

Histochemistry of the Tissue Cultures

In order to find out what type of cells were growing in the tissue cultures established from the testicular material of the propositus, the following procedures were carried out: growing cell cultures of testicular origin, and, for control, of fibroblasts from the skin and peritoneum, were trypsinized and re-cultured at varying intervals, mostly once week. Cells from monolayer outgrowths were mechanically loosened, pipetted onto slide and rapidly air-dried, whereafter the slides were used for histochemical incubation. In an alternativ procedure, the cells were grown on coverslips

which were removed from the culture bottles, washed twice and incubated as such. The activities of non-specific esterase and of number of oxidative enzymes, including that of steroid 3β-ol dehydrogenase, were demonstrated using the methods suggested by Pearse (29)

After 1 month culture (3 passages) a number of cells of testicular origin exhibited extensive tetrazolium reductase activity if either reduced NAD or dehydroepiandrosterone was used as substrate. Non-specific esterase activity was also present in most of the cells. The positively reacting cells were relatively large and roundish, and contained a centrally situated nucleus with a prominent nucleolus. There was a clear-cut histochemical difference between these cells and the fibroblast like cells from the control cultures. Undifferentiated fusiform epithelioid cells were also seen in the cultures of testicular origin but these did not show any steroid dehydrogenase activity and the other enzymatic activities were much weaker than in the more differentiated cells.

About 1 ½ months (5 passages) and 2 months (7 passages) after the cultures were set up very few cells from the testis cultures any longer differed from the control cells in their staining properties.

Our interpretation of the histochemical findings is that the majority of the cultured cells of testicular origin were derived from Leydig cells. They maintain their cytochemical properties for some time *in vitro* but later de-differentiate.

Family Data

The pedigree of the family is seen in Fig 5 There was no history of hereditary disease, stillbirths or any other abnormalities. There had been no twinning in the immediate family The patient's father had had a disease of the central nervous system, which had been tentatively diagnosed as multiple sclerosis. He had had practically no symptoms since 1959 The mother and sibs had been healthy except for minor diseases. They were not clinically investigated.

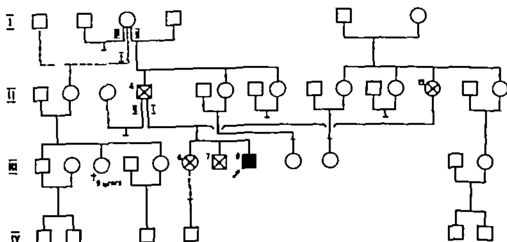


Fig 5. Pedigree of the family The propositus is indicated by an arrow The individuals marked with have been cytogenetically investigated

Cytogenetical Investigations

Oral mucosa smears were stained with haematoxylin for the determination of sex chromatin (2). Concentrated leucocyte smears were used for counting drumsticks in polymorphonuclear leucocytes. For chromosome studies, short-term cultures of leucocytes from the peripheral blood were made according to a slight modification (*) of the original method (24). Long-term tissue cultures of bone marrow (1), skin and other tissues (12) were established in the usual way and the mitoses harvested after the first or second subculture. Some hours treatment with colchicine was given before harvesting. Squashing was not performed. In all instances, the cells were brought into suspension, pipetted onto a slide and air dried. Aceto-orcein and Giemsa's stain were used. The cultures regularly yielded hundreds of mitotic plates. After screening of the slides, good metaphase figures were selected for chromosome counting. Analysis was performed by drawing or photography.

Autoradiographs of ³H-thymidine-labelled cultures of leucocytes from the peripheral blood (22) were made with the aid of Kodak AR 10 stripping film. The cultures were labelled 6 hours prior to fixation and stained with Giemsa's stain. The film was exposed for 48 hours.

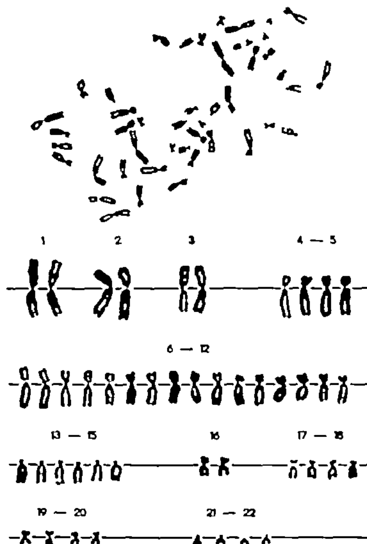


Fig. 6. Mitotic cell from culture of peripheral leucocytes of the propositus (above) and the karyotype of the same cell (below). There are 16 chromosomes in the group 6—12 and there is no Y chromosome. The karyotype is 46 XX.

Preputius 30 of buccal mucosa cells repeatedly showed sex chromatin bodies of normal size and shape. Of 2000 polymorphonuclear leucocytes studied 22 had a drumstick of normal size and shape. Small clubs were few in number Cultures of peripheral leucocytes were set up on 3 different occasions. Specimens of bone marrow skin, fascia from the abdominal wall, peritoneum, and the left testis were cultured on one occasion each. The results of the chromosome counts are seen in Table I

From the table it is evident that the modal number was 46 in all the tissues. Over 30 excellent metaphase plates from different tissues were carefully analysed Analysis invariably showed a normal female karyotype with 16 chromosomes in group C and no Y chromosome. Figs. 6–7 show examples of this karyotype. Very few polyploid cells were seen. All cells with a near-diploid chromosome number were carefully analysed They were mostly ruptured cells lacking one or a few chromosomes. In no single cell was there any evidence of a Y chromosome

Autoradiographs of cultured peripheral leucocytes labelled with H thymidine

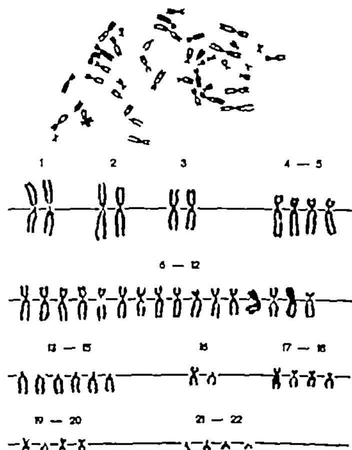


Fig 7 Mitotic II from culture of testicular origin of the preputius (above), and the karyotype of the same cell (below) The karyotype that of normal female 46, XX

Blood Grouping

Some blood groups of the propositus, his parents and sibs are shown in Table II There was nothing to suggest that the head of the family might not be the biological father of the propositus and his sibs. The Xg-grouping gives some interesting information. Since the father is Xg (a+) his only X chromosome must bear the gene for the Xg (a+) antigen. Both the X chromosomes of the mother must be Xg (a−) since she is negative. Then the propositus, who is Xg (a−) cannot have received any X chromosome from his father and thus both his X chromosomes are maternally derived.

No member of the family is colour blind.

Discussion

There is little doubt that the propositus has the karyotype 46/XX. The morphology of the chromosomes the presence of Barr bodies and drumsticks, and the late ³H thymidine labelling of one chromosome of group C support this view Undetected mosaicism might conceivably be present, but there is no evidence for this. Thus, cultured cells from 6 different tissues yielded uniformly diploid karyotypes except for a few near-diploid mostly damaged cells, and a few polyploids. We therefore think that considerable evidence points to the propositus having at present only one, 46/XX, stemline.

The clinically normal father and only brother of the propositus have larger than normal Y chromosomes in nearly all their cells. This finding is in accord with previous reports (4 23) stating that large Y chromosomes do not cause developmental disorders. It has been suggested that such large Y chromosomes exhibit only elongation or unusual spiralization and perhaps do not contain more DNA than normal Y chromosomes (32)

Cytogeneticists have stressed (10 30) that a Y chromosome is necessary for the formation of testes. Exceptions to this rule, such as the present case, and hermaphrodites with an XX complement referred to in the introduction to this paper are difficult to explain However the testicular tissue found in hermaphrodites is not infrequently associated with ovarian tissue in the same organ, the "ovotestis and rudimentary female internal sex organs are always present.

Apparently the relative importance of sex *determination* (by the genetic constitution) and of sex *differentiation* (influenced by environmental and intrinsic mechanisms) is not yet fully known.

Evidently this case differs from any that have been described before. In one of the very first papers on human chromosomes Ford *et al.* (9) reported five XX cells in a patient with Klinefelter's syndrome, but later Ford (8) suggested mosaicism in that case. Oikawa and Blizzard (28) presented a chromatin-positive child whose clinical appearance somewhat resembled that of our patient, but a probable X, isochromosome-X complement with possible mosaicism was demonstrated. Finally the 46/XX case of Shah *et al.* (31) was a pseudohermaphrodite.

There is some difficulty as to the clinical classification of our patient. His general appearance and external genitalia are male, and there is no rudiment whatsoever of female sex organs. Gonadotrophin excretion is higher than normal, as in

Table I Cytogenetical data on the propositus and his father, mother, sister and brother. Blood cultures of the propositus were made on three different occasions, which are recorded separately.

Individual studied	Tissue	Chromosome counts							Total	Karyotype
		<44	44	45	46	47	48	>48		
Propositus (III/8)	Blood	–	–	5	55	–	–	–	60	46/XX
	Blood	3	3	1	89	–	–	–	96	"
	Blood	1	–	3	56	–	–	–	60	
	Bone marrow	1	1	1	45	–	–	1	49	
	Skin	1	1	3	59	–	–	1	65	
	Peritoneum	1	–	4	54	–	–	1	60	
	Fascia	–	1	1	48	–	–	1	51	
	Testis	1	1	2	53	–	–	–	57	
Father of propositus (II/1)	Blood	4	–	3	39	–	–	–	46	46/XY (Y large)
Mother of propositus (II/15)	Blood	1	1	1	41	–	–	–	44	46/XX
Sister of propositus (III/6)	Blood	–		–	60	–	–	–	60	46/XX
	Skin			1	38	1	–	–	40	
Brother of propositus (III/7)	Blood	1	–	1	57	–	–	1	60	46/XY (Y large)
	Skin	–	–	–	40	–	–	–	40	

Table II Blood group, and secretor and PTC-taster status of the propositus, and his father, mother, sister and brother

Individual	ABO	MNS	P_1	Rh	Lu	K k	Le^a Le^b	Fy	Jk^a Jk^b	Xg	Hp	Tf	Gm Gm Gm^b	Gc	Taster	Secretor
Propositus	A_2	MNSs	+	rr	–	+	– +	–	– +	–	2–2	CC	+ – +	2–1	+	A
Father	A_1	MSMs	+	rr	–	– +	– +	+	– +	+	2–2	CC	– – +	2–1	+	A
Mother	A_2	MNSs	+	R_1		+ +	– +	–	– +	–	2–2	CC	+ – +	1–1	+	A
Sister	A_1	MNSs	+	R_1	–	+ +	– +	+	– +	+	2–2	CC	+ – +	2–1	+	A
Brother	A	MSMs	+	rr	–	+ +	– +	–	– +	–	2–2	CC	+ – +	1–1	+	A

Klinefelter's syndrome, and except for the findings of testicular histology he might be classified as a case of Klinefelter-like syndrome (3) But the slight degree of tubular sclerosis and peritubular fibrosis is not in accord with that syndrome. Furthermore, the Leydig cell hyperplasia is so marked that it cannot result from condensation of the interstices alone, but presumably reflects an absolute increase in the volume of the Leydig tissue Moreover the testes are functionally deficient. The histology of the testes, but not the clinical picture, somewhat resembles that seen in "germinal cell aplasia" (6) Clinically our patient therefore constitutes a case of hypergonadotrophic hypogonadism with some traits of Klinefelter's syndrome and some of "germinal cell aplasia We wish to underline the statement of Johnsen (21) that the classification of the various types of hypogonadism is unsatisfactory

We would like to suggest that the following events have taken place in the development of the present case: Through non-disjunction during the first or second meiotic divisions in the mother's ovary (as evidenced by Xg) a 22 A (autosomes) + XX ovum was formed The fertilization of this ovum with a normal 22A + Y sperm resulted in the formation of a 44A + XXY zygote, which began to develop. Alternatively non-disjunction with duplication of the maternal X chromosome may have taken place in one of the first cleavage divisions of a normal 44A + XY zygote. The karyotype 47/XXY is known to be male-determining, and male sex differentiation was triggered off. However during one of the very first cleavage divisions, the Y chromosome was lost through an unknown mechanism, *e g* non-disjunction and lagging at anaphase. The zygote then lost its genetic, Y borne male sex determinants, but development, once triggered off continued to form a male, and the usual features of Klinefelter's syndrome were somewhat modified by the absence of the Y chromosome.

This theory implies (i) that sex differentiation starts at a very early embryonic stage or (ii) that all Y chromosomes or cells containing Y chromosomes were lost at a later stage, which seems less probable even if selection were very strongly in favour of the XX against the XXY cells.

Since the father's and brother's Y chromosomes are larger than normal, it seems probable that the hypothetical Y chromosome of this embryo was a large one (4) It remains to be shown whether such large Y chromosomes get lost more often than normal Y chromosomes during the first or later cleavage divisions.

Our case adds a further one to those of male hypogonadism in which non-disjunction has been proved maternal by colour vision studies (26) or by determination of the Xg blood group (7 13) The suggestion by Ferguson-Smith *et al.* (7) that high maternal age is a factor in maternal non-disjunction (with the karyotype 47/XXY) does not evidently apply to all cases since in the present case maternal age at birth was 28 years (previous cases reported 39 and 41 years) This may however indicate postzygotic non-disjunction, as discussed above.

Our histochemical studies indicated that the majority of the testicular cells growing *in vitro* were probably of Leydig cell origin, since their histoenzymatic staining characteristics were similar to

those of Leydig cells *in vivo* (25) The karyotyped cells from the testicular cell cultures were thus probably of Leydig cell origin, although this has not been definitively proved. It is of particular interest to note that such androgen-producing cells showed a normal female chromosome complement. It seemed, however that these cells could not maintain their functional capacities for long *in vitro* since the histochemical reactions became weak or negative after 8 weeks culture. It is generally believed that fragments of endocrine organs must be cultured if their hormone production is to be maintained But recent observations have shown that functional activity may go on even in isolated fibroblasts (14) and thyroid cells (19) Our own observations in this case show that the adenomatoid hyperplastic interstitial tissue of the testis may maintain its cytochemical and probably even its endocrine properties for some weeks in tissue culture.

Summary

A 19-year-old male presented for treatment on account of small testes and a small penis. Biopsy of both testes revealed absence of spermatogenesis, very slight thickening of the tubular membranes and hyperplastic Leydig tissue. At laparotomy no female internal sexual organs were found. Cholecystectomy was performed because of gallstones. Clinically the patient was classified as a case of hypergonadotrophic hypogonadism with some traits of Klinefelter's syndrome and some of "germinal cell aplasia"

498 cultured cells from the peripheral blood, bone marrow skin, fascia peritoneum, and the left testis revealed a 46/XX (normal female) chromosome complement. The findings of chromosome morphology the sex chromatin pattern, and the *in vitro* labelling of mitotic chromosomes with ^{3}H-thymidine were in accord with this interpretation There was histochemical evidence that some testicular elements cultured and karyotyped were of Leydig cell origin. These cells maintained steroid 3β-ol dehydrogenase activity for about one month *in vitro* but later de-differentiated.

The father and brother of the propositus had large Y chromosomes, and the mother and sister were cytogenetically normal. Results of blood grouping indicated otherwise normal inheritance patterns, but the X-linked blood group Xg showed that both the X chromosomes of the propositus were of maternal origin.

It was suggested that the patient had originally had the karyotype 47/XXY which led to male sex differentiation, and that the Y chromosome had been lost during one of the first cleavage divisions. Other explanations are possible This appears to be the first example of a 46/XX chromosome complement in a human male

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The Leucocyte-mitogenic Effect of Serum from Rabbits Immunized with Human Leucocytes

By

RALPH GRÄSBECK, CLAS T NORDMAN AND ALBERT DE LA CHAPELLE

At present, the following materials are known to induce division or at least blast formation in peripheral leucocytes (lymphocytes) in the cell cultures used for cytogenetic studies: Phytohaemagglutinin (P.H.A.) from the bean *Phaseolus vulgaris* (13) antigens to which the leucocyte donor is sensitized (tuberculin, vaccines, pollen, etc.) (14 3 10) leucocytes of other persons (6) and finally as reported by us (4) serum from rabbits immunized with a human leucocyte concentrate.

The following hypothesis led to the preparation of the antileucocyte immune serum: P H.A. attaches itself to some structure of the leucocytes (being for instance, a leucoagglutinin) and thereby unspecifically triggers off the mitotic mechanism of the cell. Thus mitogenicity should not be a property unique t P.H.A. but should be exhibited by ny material which stimulates the same receptor structure of the leucocytes.

In order to imitate the hypothetical mode of action of P.H.A., we immunized rabbits with a human leucocyte concentrate and studied the capacity of the resulting antiserum to induce mitosis in peripheral leucocytes. These studies are now described *in extenso* As outlined in the discussion, qualitative and quantitative considerations led to the view that the leucocyte-mitogenic materials can be divided into two distinct groups, those which act as antigens and those which act by another mechanism, which apparently does not require any presensitization.

Material and Methods

Leucocyte preparations. For both the cultures and the immunizations fresh leucocytes were prepared as follows: 25 ml of heparinized blood from healthy subjects was left to stand for 1–2 hours. The plasma containing the leucocytes was sucked off and used as such for the leucocyte cultures. For the



F g 1) Typical mitotic response t antileucocyt immune serum with blasts and one mit u.



b) Negative re pons (control serum Note presence f polymo phonuclears and pycnotic nuclei

immunizations, the plasma was centrifuged for 5 minutes t 600 x g. The sedimented cells were washed three times with 25-fold volume of physiological saline, and resuspended in saline This leucocyte preparation also contained erythrocytes (about 50 per cent of he number of leucocytes) and some thrombocytes.

Leucocyte culture Hungerford's procedure (7) was used, but P H A. was omitted in the isolation of the leucocytes The culture flasks contained about 7.5 million leucocytes suspended in 1 ml of the donor plasma and 4 ml of Parker's solution T different bottles the following test substances were added: 0.05 – 0.5 ml immune serum, 0.5 ml saline containing 0.1 mg of P H A (Bacto-phytohemagglutinin P Difco batch 456657 in the early experiments and batch 461423 in later studies) and for negative control, 0.5 ml saline only 1 some series, the following materials were included: tuberculin (Purified Tuberculin from Statens Serumsinstitut, Copenhagen, Denmark) the final dilution in the culture flasks being 20 tuberculin units per ml, the effec of homologous leucocytes was studied by adding the same culture bottles two leucocyte-plasma suspensions derived from different subjects (0.5 ml of each)

Usually one of two identical culture bottles was incubated at 37 C for 3 days and the other for 4 days. Colcemid® was added 24 hours before the end of incubation. Under "Results the more positive culture is reported. In the experiments in which the time dependence of the mitotic response was studied, the incubation time varied from 1 to 7 days.

In the early experiments (4) the results were assessed as follows: *Negative result* Not more than 0.5 % mitoses and 0.5 % blasts among the leucocytes. *Positive result.* At least 5 % mitoses and 10 % blasts. *Intermediate result.* Counts between the above. *Toxic effects.* Many deformed cells and/or a gross decrease in the number of cells, and appearance of amorphous masses, i.e. lysis.

In later studies, we have counted the percentage of mitoses (500 cells were usually counted). Further the toxic effects ha been graded as follows ++ disappearance of all leucocytes except few deformed clumps, + greatly reduced number of cells, ± slightly reduced number of

cells, — no disappearance of cells compared with controls. In this gradation, ++ would correspond to the "Toxic effects" used in the early studies.

Immunization. During first immunization course five rabbits received 10^9 whole leucocytes in saline. The leucocytes were chiefly taken from one donor of blood group BRh+. However, two of the rabbits were also injected with leucocytes from donors of the blood group ORh+. This course was divided into 5 intravenous injections, given at one week intervals. The rabbits were bled one week after the last injection. From rabbit No. 2, new serum was also taken 4 months after the end of the first immunization course. Three months after the first course, the three rabbits which had received only BRh+ cells were subjected to second immunization course. The second course was identical with the first, and the same BRh+ cells were given.

For control purposes, serum samples were taken from some of the rabbits before immunization, and further one rabbit was injected with saline. Serum samples of some other rabbits were also employed. The sera were stored frozen until used. Unless otherwise stated, complement (8) and possible leucocyte-agglutination inhibitors (9, page 26) were inactivated by heating the thawed antiserum 56 C for 30 min.

The individual antisera were studied separately after the first immunization course. After the second course all the remaining old antisera were pooled with the new antisera. Of this pool the sera from the rabbits which had also received ORh+ leucocytes formed about 5 per cent.

Serological methods. Unless otherwise stated, all the following procedures were carried out at room temperature. The haemagglutinin titre was determined according to Salk (16). Aliquots of 2 per cent saline suspension of erythrocytes and saline dilution of the serum were mixed and the sedimentation pattern read after one hr. The leucoagglutinin titre of the individual antisera was assayed according to Killmann (9, page 16). However, during later experiments on pooled antiserum the titre was also assayed by modification of the method of Hartl (5): The leucocytes were isolated according to the original procedure, but since it was difficult to use the cells immediately they were stored 15 per cent glycerol at −20 C. The agglutination was assayed macroscopically. The final concentration of the leucocytes was 2×10^6/0.2 ml.

The antisera were absorbed with erythrocytes or leucocytes in the following way: The leucocytes were isolated according to Hartl (5). Erythrocytes (from the same donors) were obtained by the same procedure, but the haemolysis step was omitted. Of course this preparation contained the normal blood percentage of leucocytes. The antisera were absorbed by suspending the cells in them for one hour, whereupon the cells were removed by centrifugation.

Results

Individual cultures. The mitogenic activities of the undiluted heated antisera are given in Table I. It is seen that 4 out of 5 antisera were clearly mitogenic. The antiserum from rabbit No. 2 was at first mostly toxic (possibly owing to an exceedingly high antibody titre, cf. its high leucoagglutinin titre) but when a new serum was taken 4 months after the first immunization course, it, too, was found to be mitogenic (this was not reported in our preliminary communication) (4). The leucocytes of some subjects failed to divide in this series. However, the leucocytes of an intermediate responder (subject C) divided when treated with pooled antiserum (see below); the rest of the non-responders were not reinvestigated.

When control sera were added to the cultures, the response was negative with all sera and the leucocytes of all donors, except in one instance where again the cells from subject C gave an intermediate response.

The agglutinin titres of the individual sera are also given in Table I. The haemagglutinin titres varied from 1:2 to 1:2^{14} and the leucoagglutinin titre (Killmann technique) from 1:2 to 1:>2^8



Fig 1) Typical mit tic response t antileucocyt immune serum with blasts and one mit is.



b) Negative esponse t cont ol serum. Note presenc f polymorphonuclear and pycnotic nuclei

immunizations, the plasma was centrifuged for 5 minutes at 600 x g. The sedimented cells were washed three times with 25-fold volume of physiological saline, and resuspended in saline. This leucocyt preparation also contained erythrocytes (about 50 per cent of the number of leucocytes) and some thrombocytes.

Leucocyte culture. Hungerford' procedure (7) was used, but P.H.A. was omitted in the isolation of the leucocytes. The culture flasks contained about 7.5 million leucocytes suspended in 1 ml of the donor' plasma and 4 ml of Parker' solution. To different bottles the following test substances were added. 0.05–0.5 ml immune serum, 0.5 ml saline containing 0.1 mg of P.H.A. (Bacto-phytohemagglutinin P Difco batch 456657 in the early experiments and batch 461423 in later studies) and for negativ control, 0.5 ml saline only I some series, the following materials were included: tuberculin (Purified T berculin from Statens Seruminstitut, Copenhagen, Denmark) the final dilution in the culture flasks being 20 tuberculin units per ml; the effect of homologous leucocytes was studied by adding to the same culture bottles two leucocyte-plasma suspensions derived from different subjects (0.5 ml of each)

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In later studies, w have counted the percentage of mitoses (500 cells were usually counted) Further the toxic effects have been graded as follows: ++ disappearance of all leucocytes except few deformed clumps, + greatly reduced number of cells, ± slightly reduced number of

One control serum showed a low haemagglutinin titre. Another had a moderate leucoagglutinin titre, which disappeared after the saline "immunizations" however.

Pooled antiserum. The effect of varying amounts of heated pooled antiserum on the leucocytes of five subjects is reported in Table II. The mitogenically active dose was about 0.2–0.5 ml per flask, leucocytes of different individuals requiring varying amounts of antiserum. Larger amounts of antiserum caused lysis of the cells.

The lytic effects were deemed to be due to the presence of complement, and therefore, the antiserum had been subjected to heat treatment since the very first preliminary experiments. However, some lytic effects were observed even with heated antiserum, and this was attributed to the presence of complement in the leucocyte donor's plasma. In order to elucidate this question, in one experimental series the donor plasma was also heated. The cells of 8 ml of an ordinary leucocyte suspension in donor plasma (see *Leucocyte culture* above) were centrifuged down and washed three times with 5 ml heated plasma from the leucocyte donor whereupon they were resuspended in heated donor plasma and added to the culture flasks. Table III shows the mitotic and lytic effects observed in this experiment, together with data on the response of ordinary leucocyte cultures to unheated and heated antileucocyte immune serum and control serum. It is seen that there was no lytic effect when both the immune serum and the donor plasma had been heated. Heated and unheated immune serum exhibited mitogenic and lytic activity when ordinary culture procedures were used (unheated donor plasma) and in this particular series heating of the antiserum had no clear-cut effect.

The haemagglutinin titre of the heated antiserum pool was 1 2^{10} and the leucoagglutinin titre 1 2^{8} (Killmann technique) or 1 2 –1 2^{1} (Hartl technique) depending on whose leucocytes were used.

Table II. Comparison of mitogenic and lytic action of heated pooled antileucocyte immune serum on leucocytes of five subjects.

Amount of antileucocyte serum added to culture ml	Cultured unwashed leucocytes taken from subject									
	A		C		G		H		I	
	Mitoses	Lysis	Mitoses	Lysis	Mitoses	Lysis	Mitoses	Lysis	Mitoses	Lysis
0.5		++	0	+	9.5	+		++		++
0.4		++	9.8	+	13.2	+		++		++
0.3	5.5	+	11.0	±	10.0	±	1.8	+		++
0.2	12.6	–	5.2	–	6.6	–	3.4	+	1.8	±
0.1	1.4		0	–	0	–	0.2	–	0	–
0.05	0		0	–	0	–	0	–	0	–

(The antiserum prepared mainly against cells of subject A)

Table IV shows the mitogenic activity of the pooled antiserum after absorption with leucocytes or erythrocytes. Absorption with erythrocytes caused a moderate decrease in both agglutinin titres (the erythrocyte mass contained some leucocytes) whereas the mitogenic effect did not differ significantly from that of a control to which saline had been added instead of erythrocytes. Absorption with leucocytes, on the other hand caused a decrease in the titre of the mitogenic principle and in one series total and in another series almost total disappearance of leucoagglutinating power. However the haemagglutinin titre remained the same as that of a control.

Time-response to different mitogens. Table V shows the percentage of mitoses at different times in otherwise identical cultures containing P.H.A., pooled antileucocyte immune serum, tuberculin homologous leucocytes and rabbit control serum, respectively. It is seen that P.H.A. and antileucocyte serum gave a high percentage of mitoses and blasts after as little as 3—4 days culture, whereas tuberculin and homologous leucocytes gave lower percentages of mitoses and a maximal mitotic response later after 5—6 days.

Discussion

The outcome of this series of experiments was highly positive in that all immunized rabbits ultimately produced mitogenic antisera, whereas all control sera were inactive. The antiserum pool induced division in the leucocytes of all donors used. However there was individual variation in the response. That the leucocytes of some subjects failed to react to some individual antisera taken after the first immunization course may have been due to low antibody titres, since one of the non-responders later responded to the antiserum pool.

Table III. Mitogenic and lytic action of varying amounts of heated or unheated pooled antileucocyte immune serum on washed or unwashed leucocytes of subject A.

Serum added culture ml	Heated antileucocyt serum + leucocytes washed with heated donor plasma		Heated antileucocyte serum + unwashed leucocytes		Unheated antileucocyte serum + unwashed leucocytes		Heated control serum + unwashed leucocytes	
	Mitoses[1]	Lysis[2]	Mitoses	Lysis	Mitoses	Lysis	Mitoses	Lysis
0.5	12.8	−		++		++	0.2	−
0.4	7.2	−		++	0.2	+	0.8	−
0.3	3.8	−	5.3	+	10.0	+	0.2	−
0.2	1.4	−	12.6	−	5.2	+	0.4	−
0.1			1.4	−	4.2	+	0.6	−
0.05			0	−	0	+	0.4	−

[1] Percentage of mitoses (500 cells counted)

[2] Lysis assessed as ++ + ± − (see text)

The hypothesis leading to the preparation of the antisera was that the mitogenic principle of P.H.A. attaches itself to the leucocytes being a leucoagglutinin. The present antisera seem to act by a similar mechanism. As reported in Table IV absorption of the antiserum pool with leucocytes brought about a clear-cut decrease in the mitogen and leucoagglutinin titres, whereas a control in which absorption was performed with an equal amount of erythrocytes (containing the normal percentage of leucocytes) did not decrease the mitogenic activity and did not completely abolish the leucoagglutination (the decrease in the latter may

Table IV. Assay of haemagglutinating, leucoagglutinating and leucocyte-mitogenic activities of heated pooled antileucocyte immune serum absorbed with leucocytes or with the same volume of erythrocytes.

4.2 ml of antileucocyte serum treated with	Haemagglutinin titre[2]	Leucoagglutinin titre[2] (Hartl)	Mitoses and lysis after addition of the following amounts of treated antiserum[1]					
			0.5 ml	0.4 ml	0.3 ml	0.2 ml	0.1 ml	0.05 ml
1.5 ml saline	1:2[9]	1:2	++	++	12.6	9.2	0.6	0
erythrocytes	1:2	1:2[3]	++	++	13.8	8.6	3.2	0
leucocytes[3]	1:2[9]	no aggl.	1.2	1.4	1.2	0	0	0
0.5 ml erythrocytes[4]	1:2	1:2	++	++	++	13.4	1.4	0
leucocytes[4]	1:2	1:2	9.2	14.0	0.4	3.2	0	0

Leucocytes of subject A used for culture. Mitogenic action expressed as percentage of mitoses.
Only total lysis (++) is reported.
Cells of two subjects mixed.
Cells of a third subject.

Table V. Comparison of frequencies of mitoses and blasts in 1 to 7-day cultures of peripheral leucocytes stimulated with pooled antileucocyte serum (ALS), phytohaemagglutinin P (PHA), purified tuberculin (PT), homologous leucocytes (HL) and control serum of rabbit No. 4 (CS)

Days of culture	Percentage of mitoses					Percentage of blasts				
	ALS	PHA	PT	HL	CS	ALS	PHA	PT	HL	CS
1	0	0	0		0	0.1	1.4	0.2		0.2
2	0	2.9	0		0.1	14.2	17.1	0		0.1
3	14.0	17.2	0.3	0	0	13.4	13.2	1.2	0.2	0.8
4	10.0	18.2	0.8	0.8	0.2	31.6	40.3	2.7	0.5	1.6
5	6.5	6.7	1.4	0.7	0.3	22.1	43.7	5.7	0.3	0.8
6	1.0	2.8	0.4	2.6	0.3	4.1	31.0	1.5	0.1	5.8
7	0.4	1.1	0.4	1.0	0.1	10.2	30.8	6.3	2.3	3.8

The leucocytes used for all cultures (except the HL experiment) were from subject A. H. (a Mantoux positive). Quantities of stimulating agent added: ALS = 0.25 ml pooled serum, PHA = 100 μg, PT = 100 tuberculin units, HL = see text, CS = 0.25 ml serum. 1000 cells counted.

be due to the blood group antigens common to the erythrocytes and leucocytes)

On the basis of the present data on the frequency of mitoses, and those reported by others on blast formation (2) it can be inferred that there are differences in the mode of action of the known leucocyte mitogens. In antigen-induced cell division the percentage of mitoses is relatively low (only some clones divide?) and the mitotic response only reaches its maximum after as much as 5—6 days culture. The time response seen after the addition of homologous leucocytes was the same as that observed after addition of antigens. P H.A. and antileucocyte immune serum give a higher percentage of mitoses and act earlier the maximum mitotic response being observed after 3—4 days culture. There is no need to assume that presensitization is required.

However such an antigen theory has been put forward to explain the action of P.H.A. (3) Also, it is possible that leucocyte antigens were liberated in our rabbits and that they were present in the immune serum. However the serum from rabbit No 2, taken more than 4 months after the last injection, was active, and at such a late date one would assume that the leucocyte antigens had disappeared from the circulation. Moreover the leucocyte donor responded to the antiserum produced against his own cells. The antigen theory would then require that the donor was autoimmune. Also, there is the difference in the time-response of the leucocytes to known antigens and the immune serum. Altogether it seems very unlikely that the antiserum acts as an antigen.

We first considered the possibility that there was a difference in the mode of mitogenic action of P.H.A. and antileucocyte immune serum, since large doses of the latter causes disappearance of cells and other toxic effects, whereas the former material is mitogenic even in large doses (12) However heat treatment of the immune serum and the leucocyte donor's plasma abolished the lytic effects of the large antiserum doses and thus, in principle, there appears to be no difference between the mode of action of P H.A. and the antiserum. (Yet, this question cannot be considered as completely settled since the mitogenic action decreased somewhat and thus a possible toxic or lytic action of the antiserum may have escaped our notice.) Also, it should be remembered that the immune serum must contain numerous antibodies directed towards different antigens, including cells other than leucocytes.

As to the manner in which P H.A. and antileucocyte immune serum stimulate the leucocytes to division, the present results indicate that both contain material which attaches itself to the leucocytes. This is in line with our hypothesis that there is a receptor structure in the leucocyte which when stimulated, *e g* by attachment of agglutinins triggers off an automatic biochemical chain reaction ultimately leading to mitosis. The mitosis-triggering property of various materials would thus be unspecific, and perhaps comparable to the induction of parthenogenetic division of egg cells by simple stimuli, such as needle pricks. Possibly antigen-stimulated cell division is also brought about by a similar mechanism. The slower time-response in this case may

be due to the stimulation of fewer receptors than in the case of P.H.A. and antileucocyte serum.

It is of special interest that sea-urchin eggs are stimulated to parthenogenetic division by serum from rabbits immunized with sea-urchin eggs (15). A phenomenon which superficially resembles antibody induced mitosis of leucocytes is the enhancement of the growth of implanted tumours by anti-tumour immune serum (11).

The present results may perhaps help to explain certain autoimmune phenomena and have some bearing on theories which assume that cell growth is regulated by physiological autoantibodies (1).

Summary

Five rabbits were immunized by intravenous injection of a concentrate of whole human leucocytes. All the rabbits produced antisera which were mitogenic in cell cultures of human peripheral leucocytes. The antisera also produced lysis of cells; this effect was shown to be related to the presence of complement. Absorption of the antiserum with leucocytes removed the mitogen. Control sera were inactive. Like bean phytohaemagglutinin, the immune serum produces a high percentage of blasts and mitoses after 3—4 days' culture, whereas tuberculin and homologous leucocytes give fewer mitoses and a maximal mitotic response after 5—6 days. Phytohaemagglutinin and the immune serum probably contain substances which are attached to a receptor structure in the cell. This in turn triggers off the cell division mechanism. Their mechanism of action seems to be different from that of antigens.

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The Interrelations of Erythroagglutinating, Leucoagglutinating and Leucocyte-mitogenic Activities in *Phaseolus vulgaris* Phytohaemagglutinin

By

Clas T. Nordman, Albert de la Chapelle and Ralph Gräsbeck

In order to achieve division of peripheral lymphocytes *in vitro* it is necessary to add a mitogen to the cultures (18). The routinely employed mitogen is phytohaemagglutinin (PHA), a protein panerythroagglutinin[1]) preparation from *Phaseolus vulgaris* (21). The question has been raised whether its erythroagglutinating and mitogenic activities could be separated (3). In a preliminary communication we reported that such a separation had been accomplished by absorbing PHA with erythrocytes, but not by chemical methods (5). These studies are now described *in extenso*. Meanwhile, similar findings have been reported by others (2).

As to the action of other mitogens on lymphocyte cultures, there appears to be a difference between the mitogenic actions of PHA and antigens (6). Our group found that the stimulation exerted by anti-leucocyte immune serum is of the PHA type and that the active principle in the antiserum appears to be a leucoagglutinin (8, 9). Therefore we investigated whether PHA had any leucoagglutinating capacity. The relation of erythroagglutinating, leucoagglutinating and leucocyte-mitogenic activities to each other was analysed by absorbing PHA with erythrocytes and leucocytes followed by elution. These studies are also reported here.

[1]) To avoid confusion we use the term erythroagglutination instead of haemagglutination to denote agglutination of erythrocytes.

Material and Methods

Starting material. Bacto-phytohemagglutinin P batch No. 434037 was used in the chemical, and batch No. 456637 in the serological experiments. They were prepared according to Rigas *et al.* (21) by Difco Laboratories Inc.

Chemical methods. Protein was assayed from the effluents by reading the optical density at 280 nm (1 mg PHA/ml saline gave the reading 0.6). The other solutions were assayed by the method of Lowry *et al.* (16). All preparative work was done at +4° C. *Ion exchange chromatography* was performed with DEAE-cellulose and CM-cellulose columns (size 23 cm, 10 ml fractions collected) mainly according to Gräsbeck *et al.* (7). In the DEAE-cellulose chromatography 5 mM phosphate pH 9.0 was used as equilibration buffer and 0.5 M phosphate pH 3.0

as the last solution in gradient of falling pH and rising concentration. Finally the column was eluted with 0.5 M phosphoric acid. CM-cellulose chromatography was performed with 5 mM phosphate pH 5.5 as equilibration buffer and the concentration of phosphate and the pH in the solutions used for elution was varied stepwise to 0.1 M and pH 7.35. *Gel filtration* was performed in physiological saline, using Sephadex G-200 (3×47 cm, 5 ml fractions). *Starch gel electrophoresis* was performed by the vertical technique of Smithies (24) with borate buffer pH 8.6. A 6 /cm current was applied for 18 hrs. 1–5 mg of protein was pplied to gel with cross-section of 0.5×1 cm. After the runs, the gel was stained with amido black or cut int 50 pieces 0.5 cm wide, from which fluid was expressed by freezing followed by thawing and centrifugation through nylon filter cloth (0.1×0.1 mm mesh, Schweiz. Seidengazefabrik A.G. Zürich). After protein measurement, the peaks were pooled. *Immuno-electrophoresis* was performed according to the micro method of Scheidegger (25) with the pH 8.6 buffer described by Hirschfeld (11). The antisera were prepared by immunizing rabbits intramuscularly with 25 mg PHA, divided int 10 injections, the first injections with Freund's complete adjuvant, the later ones without.

All fractions to be tested were dialysed before the erythroagglutinin assays, which were performed according to the method of Salk. Before they were added t the leucocyte cultures, they were also sterilized by filtration through Seitz filters.

Serological methods. The *erythroagglutinin titre* was determined by adding 0.1 ml of 1–2 per cent suspension of washed red cells to 0.1 ml of the solution to be assayed or twofold dilution series. The titre was recorded as the greatest dilution giving clear-cut positive sedimentation pattern (22). The *leucoagglutinin titre* was assayed according to Killmann (14) (however without adding acetic acid to haemolyse the red cells) and occasionally according to Hartl (10). The same volumes were used as in the erythroagglutination procedure. In Killmann procedure equal amounts of erythrocy-



Fig. 1. a) Mixed agglutination. Erythrocytes and leucocytes in the same clump. b) Separate agglutination of leucocytes and erythrocytes. The clumps contain only one kind of cell.

tes and leucocytes are present, and we observed that PHA in higher concentrations gives *mixed agglutination* of erythrocytes and leucocytes in the same clump (Fig 1). In lower concentrations the leucocytes and erythrocytes form separate clumps uncontaminated with the other cell type (Fig 1 b). In still lower concentrations there is no agglutination of leucocytes whereas the erythrocytes still agglutinate. This erythroagglutinin titre was also recorded. The reading was done macroscopically because rouleaux formation made microscopic end point determination difficult. All agglutination assays were made 37° C and with B Rh positive cells from the same donor.

Repeated *absorption* of PHA was done with fresh red cells from heparinized blood, washed four times with fivefold volume of saline. PHA was incubated at 37°C for 30 min. with packed erythrocytes and shaken by hand every 10 min. After centrifugation sample was taken for examination and the rest incubated in the same way with washed erythrocytes.

For comparative absorption of PHA with erythrocytes and leucocytes and for elution experiments total of 1600 ml one-day old citrate blood from four 0 Rh positive donors was used. The leucocyte concentrate was prepared according to Hartl (10) and the erythrocytes by the same procedure, but with the haemolysis step omitted. The washed erythrocytes contained 1–2 leucocytes per 1000 cells. The leucocyte concentrate contained 1–2 erythrocytes or ghosts per 200 cells. The ratio of polymorphonuclears to mononuclears was about 3 1 For absorption, PHA was incubated at 37°C for 60 min. and centrifuged. To evaluate possible destructive action of liberated leucocyte enzymes on PHA, PHA was added to one half of the supernatant remaining after leucocyte absorption and incubated once more.

To study what activity could be eluted from the cells used in the absorptions, the cells from the preceding experiments were treated with an excess of PHA. They were washed three times with 12-fold volume of saline and resuspended in saline.

Table I. Comparison of erythrocyte leucocyte and mixed agglutination titres of PHA. In most cases two extreme values are reported.

Methods of assay	Dilution and corresponding concentration															
	1	2	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]	2 [illegible]
	2000	1000	500	250	125	60	30	15	8	4	2	1	0.5	0.25	0.125	0.06 µg/ml
Erythroagglutination with the Salk method	+	+	+	+	+	+	+	±	−	−	−	−	−	−	−	−
	+	+	+	+	+	+	+	+	+	±	−	−	−	−	−	−
Leucoagglutination with the Hartl method	+	+	+	+	+	+	+	±	−	−	−	−	−	−	−	−
Agglutination with the Killmann method:																
pure leucoagglutination				±	±	+	+	+	+	±	−	−	−	−	−	−
						±	±	+	+	+	+	+	±	±	−	−
pure erythroagglutination				±	±	+	+	+	+	+	+	±	±	−	−	−
						±	±	+	−	+	+	±	±	±	±	−
mixed agglutination[1]	+	+	+	±	±	−	−	−	−	−	−	−	−	−	−	−
	+	+	+	−	+	±	±	−	−	−	−	−	−	−	−	−

1 the same tubes.

as the last solution in gradient of falling pH and rising concentration. Finally the column was eluted with 0.5 M phosphoric acid. CM-cellulose chromatography was performed with 5 mM phosphat pH 5.5 as equilibration buffer and the concentration of phosphate and the pH in the solutions used for elution was varied stepwise to 0.1 M and pH 7.35 *Gel filtration* was performed in physiological saline using Sephadex G-200 (3 × 47 cm, 5 ml fractions) *Starch gel electrophoresis* was performed by the vertical technique of Smithies (24) with borate buffer pH 8.6. A 6 /cm current was applied for 18 hrs. 1—5 mg of protein was applied to gel with cross-section of 0.5 × 1 cm. After the runs, the gel was stained with amido black or cut into 50 pieces 0.5 cm wide from which fluid was expressed by freezing followed by thawing and centrifugation through nylon filter cloth (0.1 × 0.1 mm mesh, Schweiz. Seidengazefabrik A.G., Zurich) After protein measurement, the peaks were pooled. *Immuno-electrophoresis* was performed according to the micro method of Scheidegger (23) with the pH 8.6 buffer described by Hirschfeld (11) The antisera were prepared by immunizing rabbits intramuscularly with 25 mg PHA, divided into 10 injections, the first injections with Freund's complete adjuvant, the later ones without.

All fractions to be tested were dialysed before the erythroagglutinin assays, which were performed according to the method of Salk. Before they were added to the leucocyte cultures, they were also sterilized by filtration through Seitz filters.

Serological methods. The *erythroagglutinin titre* was determined by adding 0.1 ml of 1—2 per cent suspension of washed red cells to 0.1 ml of the solution to be assayed or twofold dil tion series. The titre was recorded as the greatest dilution giving clear-cut positive sedimentation pattern (22) The *leucoagglutinin titre* was assayed according to Killmann (14) (however without adding acetic acid to haemolyse the red cells) and occasionally according to Hartl (10) The same volumes were used as in the erythroagglutination procedure. In Killmann's procedure equal amounts of erythrocy



Fig. 1) Mixed agglutination Erythrocyt and leucocyt in the same clump. b) Separat gglut nation f l ucocytes and erythrocytes Th l mps contain only one kind f cell.

additional cathodic and two additional anodic lines were obtained with PHA.

Gel filtration of PHA in Sephadex G-200 gave only one peak possessing both activities. Qualitative assay of erythroagglutinating and mitogenic activities in the fractions emerging from the Sephadex column did not indicate any displacement of the activities in relation to each other only trailing of inactive proteins being observed.

Serological studies Table II shows the effect of repeated absorptions of PHA with washed erythrocytes. The erythroagglutinating activity (also determined with the Killmann procedure) disappears after the first absorption, but leucoagglutinating and mitogenic activities remain. However there is a decrease in the total leucoagglutinin titre after the first absorption. This decrease is of the same magnitude (3 tubes) as that observed in the titre of mixed agglutination. The slow decrease in leucoagglutinin and mitogenic titres seen after repeated absorptions may be due to small amounts of leucocytes present among the red cells or to dilution in the erythrocyte volume.

From Table III t is evident that leuco-

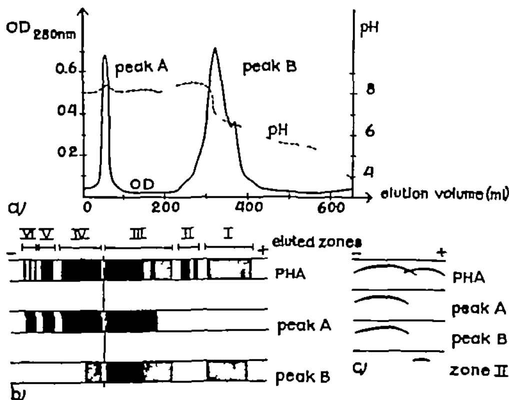


Fig 2.) DEAE-cellulose chromatography of PHA (100 mg). Peaks A and B gave the same erythroagglutinin and mit genicity titres as the original PHA b) Starch g l electrophoresis f PHA and peak A and B from DEAE-c llul hromat graphy The erythroagglutinin and mitogenic act vities were both ontained in zones III and IV The other ones lacked both activities) Immuno-electrophore is f PHA peaks A and B from DEAE-c llul hromatography and zone II from starch g l electrophoresis.

cytes, in contrast to erythrocytes, absorb both leucoagglutinins and mitogenic activity. The leucocytes also absorb erythroagglutinating activity. These results could be due to destructive action of some substances liberated from the leucocytes. To exclude this possibility we added 1/4 and 1/16 of the originally used leucocyte volume to the same amount of PHA and studied whether the PHA activity was destroyed. These experiments gave the expected result, i.e., all titres decreased less when smaller amounts of leucocytes were used. Another control was made by adding PHA to half the leucocyte treated PHA solutions followed by renewed incubation. No clear-cut decrease in any titres was obtained.

Elution of washed erythrocytes and leucocytes (treated with an excess of PHA) with heat or pH adjustments liberated about the same amounts of erythroagglutinins, leucoagglutinins and mitogenicity from the two cell types (Table IV). The only significant difference was that all the leucoagglutinating activity eluted from the erythrocytes was of mixed agglutination type, whereas the leucocyte eluate showed the same difference (1:2[illegible]) between the total leucoagglutination and mixed agglutination titres as found for the original PHA (cf. Table I).

When the leucoagglutinin titres were assayed with the Hartl method after the above absorptions and elutions, the relative changes in the leucoagglutinin titres were the same as those observed with the Killmann technique and reported in Tables III and IV.

Discussion

The chemical investigations of PHA show that the erythroagglutinating and mitogenic factors are identical or chemically very similar. The fact that the two chromatography peaks gave the same titres for erythroagglutinating and mitogenic activities and the results of the immuno-electrophoretic studies made us suspect that the resolution into two peaks achieved by DEAE-chromatography was

Table II. Erythroagglutination, leucoagglutination and mitogenicity titres after repeated absorption of PHA solution with washed erythrocytes. After every absorption 1.0 ml was taken for tests.

Treatment	Titres			
	Erythroagglutination (Salk)	Leucoagglutination (Killmann) mixed aggl.	total aggl.	Mitogenicity[1]
Control 1.0 mg PHA/ml saline	1:2	1:2[illegible]	1:2	1:2
10 mg PHA/10 ml + 5.0 ml erythr.	none	none	1:2[illegible]	1:2
9.0 ml of preceding supernatant + 5.0 ml erythr.			1:2	1:2
8.0 ml			1:2[illegible]	1:2[illegible]
7.0 ml	–		1:2[illegible]	1:2[illegible]
6.0 ml			1:2[illegible]	1:2[illegible]
5.0 ml	–	–	1:2[illegible]	1:1

[1] 0.3 ml of solution   be tested was added per culture.

an artefact. However since the same resolution was seen after CM-cellulose chromatography and the two peaks gave different patterns in starch gel electrophoresis, a real fractionation of the active proteins must have occurred. DEAE-cellulose chromatography of *Ricinus communis L.* extracts also results in several erythroagglutinating fractions (25) Our results suggest that the erythroagglutinating and mitogenic factors are contained in the cathodic protein line in the immunoelectrophoretic patterns corresponding to zones III and IV in starch gel electrophoresis. Also an erythroagglutinating cathodic and a non-erythroagglutinating anodic line have been found in immunoelectrophoresis of potato extracts (15)

Table III Erythroagglutination, leucoagglutination and leucocyte-mitogenicity titres of PHA after absorption with erythrocytes and various amounts of leucocytes (with control of destructive action of leucocytes on PHA)

2.0 mg PHA in 4.0 ml saline treated with	Titres[1]		
	Erythroagglutination (Salk)	Leucoagglutination (Killmann)	Mitogenicity
A. nothing	$1:2 - 1:2^{6}$	$1:2^{4} - 1:2^{7}$ [illegible])	$1:2^{8}$
B. erythrocytes 2.0 ml	none	$1:2^{8} - 1:2$ [illegible])	$1:2^{8}$
C. leucocytes 2.0 ml	none – 1:1	none – 1:1	none
D. 0.5 ml	$1:2^{4}$	1:2 – 1:2	none
E. 0.125 ml	1:2	1:2 – 1:2	$1:2^{3}$
F 1.0 mg PHA added to 2.0 ml supernatant of C	1:2	$1:2 - 1:2^{4}$	$1:2^{3} - 1:2^{4}$
G. – – of D	$1:2^{6}$	1:2 – 1:2	$1:2^{8}$
H. – – of E	$1:2^{6}$	$1:2 - 1:2^{4}$	1 > 2

Two assays were made, one before, the other after the determination of the mitogenicity titre. Only if they gave different results are both reported.
See footnote to Table II.
Mixed agglutination titre $1:2^{3}$
No mixed agglutination.

Table IV Elution of PHA-treated erythrocytes and leucocytes and assay of the erythroagglutinating, leucoagglutinating and leucocyte-mitogenic action of the eluate. 20 mg PHA was absorbed with 2.0 ml erythrocytes or leucocytes. After washing of the cells, 2.0 ml saline was added, followed by elution by heat or pH adjustments

Cells eluted	Method of elution	Titres			
		Erythroagglutination (Salk)	Leucoagglutination (Killmann)		Mitogenicity
			mixed aggl.	total aggl.	
Erythrocytes	heat	1:2	1:2 – 1:2	1:2 – 1:2	1:2
– –	pH	$1:2^{6}$	1:2 – 1:2	1:2 – 1:2	1:2
Leucocytes	heat	1:2	1:2	$1:2^{6}$	$1:2^{3} - 1 > 2^{8}$
– –	pH	$1:2^{3}$	$1:2 - 1:2^{8}$	$1:2^{8}$	1:2

See footnote [illegible]) Table III.
See footnote to Table II.

In contrast to our results, Punnet *et al* (19) claim to have succeeded in a chemical separation based on fractional precipitation

On the basis of the present serological studies we have formed the following opinion regarding the composition of PHA. PHA contains both a pure leucoagglutinin and an erythroagglutinin. In higher concentrations the latter also causes agglutination of leucocytes and gives mixed agglutination (The explanation for the mixed agglutination may be that there are common antigens in erythrocytes and leucocytes) (26) The mitogenicity of *PHA* is due to its leucoagglutinating capacity The pure leucoagglutinin and the leucoagglutinating capacity of the erythroagglutinin together determine the total leucoagglutinating power of PHA and thus its total mitogenicity

The following data support this view: Repeated absorption of PHA with erythrocytes removes all its erythroagglutinating activity but leaves the leucoagglutinating and mitogenic factors in the supernatant (Table II) However the first absorption with erythrocytes decreases the leucoagglutinin titre more than the subsequent absorptions (Tables II and III) The explanation for this phenomenon is apparently that the leucoagglutinating capacity of the erythroagglutinin (*i.e.* the mixed agglutination capacity) is removed Leucocytes remove the leucoagglutinating and mitogenic factors and also the erythroagglutinin (Table III) Elution of PHA-treated erythrocytes gives an extract having pure erythroagglutinating activity which higher concentrations gives mixed agglutination but lacks pure leucoagglutinating activity This eluate is also mitogenic (Table IV) and the leucoagglutinating capacity of the erythroagglutinin seems to be responsible for this. Elution of leucocytes gives erythroagglutinin (with mixed agglutination activity) pure leucoagglutinin and mitogenic activity (Table IV) This result is quite in line with the effect seen when PHA is absorbed with leucocytes (Table III) Some data suggest that the mitogenic titre of the erythroagglutinin may be lower than that of the pure leucoagglutinin (*cf* Tables II–IV)

The Killmann leucoagglutination test may be criticized referring to Bakemeier *et al* (1) who argue that erythrocytes coated with antibody may cause an unspecific clumping of leucocytes. Although we used an ordinary light microscope (× 1250, no front lens in the condenser), we are sure that there were no erythrocytes in the pure leucocyte aggregates, because the erythrocytes are clearly visible in the mixed agglutination clumps. The mixed agglutination can hardly be due to such an unspecific effect, because we have eluted almost the same quantity of erythroagglutinin from the leucocytes as from the same volume of erythrocytes (Table IV) Further the additive effect of the two leucoagglutinating capacities argues against an unspecific mixed agglutination (Tables II and III)

If the present view regarding the composition of PHA is accepted it becomes understandable that we did not succeed in separating the erythroagglutinating and mitogenic activities by chemical means It should also be noted that after these chemical procedures the erythroagglutinating fractions were mostly subjected to tests of mitogenicity and usually not *vice versa*.

Our present view is in agreement with that of Hirschhorn *et al* (12 13) who (without providing experimental data) suggest that PHA is a leucoagglutinin.

Summary

Chemical investigations were performed on a commercial preparation of *Phaseolus vulgaris* phytohaemagglutinin using ion exchange chromatography gel filtration, starch gel and immuno-electrophoresis. PHA was found to contain several proteins, but separation of its erythroagglutinating and leucocyte mitogenic activities was not achieved

After repeated absorptions of PHA with erythrocytes, mitogenic and pure leucoagglutinating activities remained in the superantant. Leucocytes absorb both erythroagglutinating leucoagglutinating and mitogenic activities. From erythrocytes and leucocytes all the three activities could be eluted in about equal amounts, but the leucoagglutinin eluted from erythrocytes gave only mixed agglutination whereas the leucoagglutinin from leucocytes gave the same degree of pure leucoagglutination as PHA. No indications were found of the existence in PHA of a pure erythroagglutinin lacking mitogenic activity It seems that PHA contains a pure leucoagglutinin and an erythroagglutinin, which also agglutinates leucocytes. Mitogenicity is caused by both because of their leucoagglutinating power

Acknowledgements

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Differences in the Requirements of Normal and Pernicious Anaemia Leucocytes in Cell Culture

By

Albert de la Chapelle and Ralph Gräsbeck

The megaloblast phenomenon occurring in human vitamin B deficiency is thought to be due to blocked formation of deoxyribonucleic acid (DNA) concomitant with normal rates of ribonucleic acid (RNA) synthesis and cytoplasmic growth (1 10) Studies by several authors (4 8, 11 12) on the micro-organism *Lactobacillus leichmannii*, which exhibits "unbalanced growth" and macro forms when grown in medium deficient in vitamin B, indicate that this phenomenon is due to impaired DNA synthesis, in which process vitamin B is needed as a cofactor in the reduction of ribosyl to deoxyribosyl groups. There is some evidence (1 8) that analogous step occurs in the biosynthesis of DNA mammalian cells. The same block that leads to unbalanced growth in *L. leichmannii* has therefore been assumed to be the cause of megaloblastosis and analogous phenomena in other tissues.

In view of this, our finding (3) that peripheral leucocytes of pernicious anaemia patients grow and divide at a normal rate in a vitamin B_{12}-deficient medium was somewhat unexpected.

In our report (3) we mentioned that very simple inorganic salt solutions were sufficient as culture media in the short term cultures used. Later our attention was drawn to the fact that in another laboratory (7) peripheral leucocytes from normal individuals failed to divide in such poor media. It appeared possible, at least, that these findings were not incompatible with each other and that the ability of the cells to divide in poor media might be an exclusive property

of cells from pernicious anaemia patients. To test this hypothesis, the experiments reported in this paper were performed.

Material and Methods

Two male and six female patients with pernicious anaemia were studied. Before treatment they all showed severe hyperchromic anaemia, increased red cell diameter megaloblastic bone marrow peripheral leucopenia and hypersegmentation of the granulocytes. The Schilling test gave typical results in all cases and the serum vitamin B_{12} content was abnormally low. Treatment with vitamin B_{12} was followed by reticulocytosis and clinical remission. Samples of blood from 9 normal individuals or patients with diseases other than pernicious anaemia, including one case of sideropenic anaemia, were used as controls.

Our culture technique was slight modification (2) of Moorhead' (9) original method. Peripheral blood was withdrawn into heparin and the leucocytes were separa ed from the erythrocytes by sedimentation and slow centrifugation. The suspension of viable leucocytes in the patient's own plasma was then used for preparing leucocyt cultures. On every occasion, 6–12 flasks were set up and incubated. Flasks with different media were harvested after 3, 4 and 5 day culture. Six hours prior fixation, demethylcolchicine (Colcemid®) was added in order to cause accumulation of mitoses. The final medium in which the leucocytes were grown consisted of:

) 20 of the patient own plasma,
b 80 culture medium,
small amount of phytohaemagglutinin, and
d some heparin

Three different cul ure media were used:
Parker solution containing inorganic salts, amino acids, purines, vitamins, glucose, d-ribose ... holesterol, penicillin and streptom in,
b *Hanks* solution containing inorganic salts and glucose and
( ) *Dulbecco* solution containing ... al only

Evaluation of the results After 3 5 ... tion, positiv cul re is recogn

presence of numerous mitoses and of large light cells with nucleoli ("blast" cells) and the disappearance of granulocytes. "Negative" cultures contain only one or two occasional mitoses and practically no blast cells, whereas the numerous small lymphocytes and granulocytes remain virtually unchanged. Intermediat results are seldom encountered when phytohaemagglutinin is used as mitogenic stimulus. Counts of mitoses and blast cells do not usually yield satisfactory results, since there is often non-random distribution of the different cell types on the slides. Therefore the evaluation is based on the general appearance of the preparations, it can be arrived at after few glances (Fig. 1)

Results

Parker's solution invariably yielded positive results. The results obtained with Hanks and Dulbecco s solutions are seen in Tables I II Typical examples of positive and negative cultures are seen in Fig 1 It was evident that cells from normal subjects did not divide if Dulbecco's solution was used. Leucocytes from all except one of the patients with untreated pernicious anaemia did grow and divide in this medium. As soon as clinical remission had been achieved with vitamin B_{12} treatment, the leucocytes behaved like those of normal subjects In contrast, all cells studied grew in Hanks solution.

In order to elucidate whether a factor in the patient's plasma might be responsible for the ... acity of pernicious ... in glucose ... d ... experiment ... solution. ... patients ... o ... ncubated ... and ... the ... ent ... n,

Table I. Growth of peripheral leucocytes of 9 normal subjects. + indicates growth and − absence of growth after 3−5 days' incubation. Hanks' and Dulbecco culture media were used.

Case No.	Hanks	Dulbecco
1	+	−
2	+	−
3	+	−
4	+	−
5	+	−
6	+	−
7	+	−
8	+	−
9	+	−

Table II. Growth of peripheral leucocytes of 8 patients with pernicious anaemia before and after parenteral treatment with vitamin B_{12}. The treatment includes Schilling tests. + indicates growth and − absence of growth after 3−5 days' incubation. An intermediate result is registered as ±. The culture media of Hanks and Dulbecco were used.

Case No.	Hanks: Before treatment	Hanks: After treatment	Dulbecco: Before treatment	Dulbecco: After treatment
1	+	+	+	−
2	+	+	+	−
3	+	+	+	−
4	+	+	+	−
5	+	+	+	−
6	+	+	+	−
7	+	+	+	−
8[1]	+	+	±	

The patient had received 1 mg vitamin B_{12} (Schilling test) 14 hrs prior to this experiment.

After 10 days' treatment an intermediate result was obtained, but 10 days later, when full clinical remission had been achieved, the culture was negative.

This patient did not have the clinical features of full-blown pernicious anaemia, i.e. the bone marrow was not clearly megaloblastic.

and a few mitoses were seen. Normal cells grown in plasma from the patients with pernicious anaemia yielded entirely negative results. Although the experiments were not quite conclusive, they gave the expected result that no factor promoting growth in the absence of glucose was present in the plasma of the patients.

Since there is a slight difference in inorganic salt composition between Hanks' and Dulbecco's solution, we also added glucose to Dulbecco's solution. It was shown that this mixture behaved like Hanks' solution.

In our experience, cultures harvested after 3, 4 and 5 days' incubation in Hanks' solution normally show considerable differences. After 3 days, there is usually a large proportion of blasts and few mitoses, but small lymphocytes and occasional granulocytes are seen. At 3 ½−4 days there is a wave of mitoses. Blast cells are numerous, and the number of small lymphocytes is reduced. Practically no granulocytes remain at this stage. At 5 days very few mitoses occur and the predominant cell is blast-like. The preparations obtained in this study form no exception to this pattern. In the Dulbecco cultures of cells from untreated patients with pernicious anaemia, there was regularly a slightly smaller proportion of blast cells and mitoses than in the cultures with Hanks' solution. However, there was no trend towards slow growth, i.e. the 5-day cultures did not show more mitoses than those harvested one day earlier.

Discussion

It is evident that cells from cases of untreated pernicious anaemia grow and

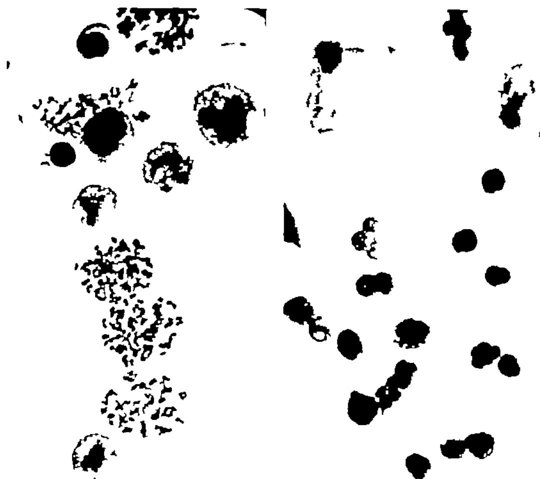


Fig. 1. The left figure depicts a typical view of a positive culture. Note the presence of mitoses, large light blast-like cells with nucleoli and the absence of granulocytes. In the right figure a detail of a negative culture is seen. Note the presence of granulocytes, numerous small lymphocytes and some cell debris. The two pictures were taken with the same technique and at the same magnification.

divide even in Dulbecco's solution which contains inorganic salts only. However, leucocytes from healthy persons and patients with disorders other than pernicious anaemia fail to grow in this solution, but do grow in Hanks solution. The same is true of cells from treated pernicious anaemia patients. The only relevant difference between the two media is that Hanks solution contains 1 g glucose per litre. We therefore conclude that there is a difference between the peripheral leucocytes of normal subjects and those of pernicious anaemia patients. This difference might be in some way analogous to that between normoblasts and megaloblasts. The latter contain a substantially increased amount of RNA and cytoplasm per cell (1, 10). Thus the peripheral lymphocytes of the pernicious anaemia patients may also contain somewhat more cytoplasmic material than

those of normal persons. This extra material may provide the pernicious anaemia lymphocytes with the energy and material required in the mitotic process which normal cells have to obtain in the form of supplementary glucose. After treatment with vitamin B_{12} this property is rapidly lost.

The fact that leucocytes from pernicious anaemia patients divide in a vitamin B_{12} deficient medium, as shown in our previous work (3) deserves some further comments. Why are these cells able to replicate their DNA? Since the leucocyte donors suffered from overt megaloblastosis it appears unlikely that the exceedingly small amounts of vitamin B_{12} present in the cultures would be sufficient for the synthesis of DNA, anyway not at a normal rate. One possible explanation, however is that the dividing lymphocytes reutilize DNA or deoxyribosyl groups from the destroyed granulocytes, thus obviating their need for vitamin B_{12}. It is widely accepted among cytogeneticists that the granulocytes disappear from growing lymphocyte cultures but remain unchanged if the lymphocytes do not grow

It also occurred to us that the mere destruction of the granulocytes with liberation of their nuclear material might act on the lymphocytes as a mitogenic stimulus. In two experiments leucocytes destroyed with the aid of a mechanical homogenizer and by ultrasound, respectively failed to exert mitogenic action on lymphocytes from the same test subjects. These experiments indicate that the components of the disappearing granulocytes do not at least primarily initiate growth. A further trigger mechanism (*e g* phytohaemagglutinin) is necessary for induction of growth (5)

Our results further indicate that Parker's solution, a very rich and expensive medium universally used for short term *in vitro* culture of peripheral leucocytes, is not at all necessary Hanks solution, which contains inorganic salts and glucose only is quite as good a medium for this purpose.

Summary

Using ordinary methods for *in vitro* culture of leucocytes from human peripheral blood, it was shown that leucocytes from seven out of eight pernicious anaemia patients tested grew and divided in Dulbecco's solution which is composed of inorganic salts only Leucocytes from nine normal persons and subjects with disorders other than pernicious anaemia failed to grow in this medium, but grew in Hanks solution which consists of inorganic salts and glucose. The ability of pernicious anaemia leucocytes to grow in a glucose-deficient medium was lost when the leucocyte donors had been brought into haematological remission by vitamin B_{12} treatment. It is suggested that the cytoplasm of lymphocytes from pernicious anaemia patients contains material which enables them to divide in a glucose-deficient medium. The reason why pernicious anaemia leucocytes are able to divide in a vitamin B_{12} deficient medium may be utilization of deoxyribotides from autolysing granulocytes.

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Calculations on the Dynamics of Vitamin B_{12} in Fish Tapeworm Carriers Spontaneously Recovering from Vitamin B_{12} Deficiency

By

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Fish tapeworm anaemia can be considered a unique phenomenon. The presence of the worm in the intestine causes an almost selective malabsorption of vitamin B_{12} in its host, while the absorption of other nutrients is practically undisturbed. The same, of course, can be said of genuine pernicious anaemia, but, in contrast to those suffering from the latter disease fish tapeworm anaemia patients are otherwise normal and experience spontaneous haematological remission after expulsion of the worm (1, 2).

In the present study the only treatment the subjects received was dosage with anthelmintics. After the expulsion of the worm the serum vitamin B_{12} concentrations were followed for a considerable time. Mathematical analysis of the data obtained affords interesting insight into the turnover of vitamin B_{12}. Earlier turnover calculations were based either on subjects with decreasing vitamin B_{12} reserves (e.g. postgastrectomy patients) or on tracer studies, in which uniform mixing of the isotope with the endogenous vitamin is open to doubt (for references and discussion, see references 3, 4, 5). In contrast to these, the present calculations are based on spontaneously increasing body stores.

Material and Methods

The study was made on a series of ten tapeworm anaemia patients with severe vitamin B_{12} deficiency. One patient (No. 10) had coronary disease and was suffering from pneumonia on admission to hospital. The others showed no signs of diseases other than the vitamin B_{12} deficiency and the tapeworm infection.

The clinical and haematological tests performed are shown in Table I.

The serum vitamin B_{12} concentrations were assayed microbiologically with *Euglena gracilis* of the z strain according to the method of Hutner, Bach and Ross (7).

The parasites were expelled with 3–4 grams of *Extractum filicis* administered orally. Three of the

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patients (Nos. 5 7 and 8) were given blood transfusions of one bottle of whole blood (300 ml) before the tapeworm cure, and one patient (No. 1) received one bottle of whole blood before and two bottles after expulsion of the worm. Otherwise, apart from removal of the parasite, no treatment was given.

After the expulsion of the worm the blood picture reticulocytes and serum vitamin B_{12} concentrations were followed. After recovering satisfactorily the patients were allowed to leave the hospital and the follow-up examinations were performed t the out-patient department. The follow-up period varied from 6 to 36 months, the average period being 20 months.

Results

The worm cure was successful in all cases and no tapeworm ova could be detected at re-examination.

As seen from Table I the serum vitamin B_{12} concentrations before the worm cure were below 50 pg/ml (average 15 pg/ml) All but one of the patients had anaemia (Hb<11 0 g%) Even in this case (No 2) the bone marrow was megaloblastic and the serum vitamin B_{12} level was as low as 16 pg/ml

The results of the haematological examinations are also shown in Table I The correction of the anaemia took about two months which is consistent with earlier findings (1 2) and identical with the time required for achievement of remission in genuine pernicious anaemia patients receiving vitamin B_{12} therapy

In Figs. 1 and 2 the serum vitamin B_{12} concentrations of the patients studied are plotted against time (for practical reasons on a semilogarithmic scale) Ten days after the worm cure all the vitamin B_{12} values were still below 50 pg/ml and 100 days after the cure they were between 40 and 100 pg/ml. It took 400 days for all the values measured to exceed 100

Table I Haematological and some other data before and after worm expulsion.

Patients[1]			pH of gastric juice after Histalog stimulation	Serum vit. B_{12} concentration pg/ml	Maximal reticulocyte response		Haemoglobin, erythrocyte count and me[an] corpuscular haemoglobin							
							Before worm expulsion			3-7 weeks later			4-8 months [later]	
No.	Age years	Sex			Days after worm cure	Reticulocytes, per cent	Hb	RBC	MCH	Hb	RBC	MCH	Hb	RBC
1	34	F	6.0	17	6	10.5	6 0	1 70	34	12.1	3.54	34	12.9	3.90
2	73	F		16	–	–	12.5	2.80	45	12.5	3.22	39	14.5	4.56
3	56	M	6.5	24	6	14.3	6.7	1.98	34	9.9	3.28	30	13.1	4.67
4	59	M	3.3	5	6	10.8	6.6	1.09	39	11 7	3.86	30	–	–
5	54	F	2 5	29	–	–	10.8	3 44	32	11.5	3 85	30	–	–
6	54	F	7.5	< 5	8	11.2	5.1	1.20	43	11.0	3.66	30	11 7	4.30
7	53	F	7 0	11	5	4.0	6.0	1.52	40	12.3	3.98	31	–	–
8	19	F	3.9	25	8	12.3	5.7	1 48	39	8.7	–	–	12.3	–
9	63	F	1 7	14	8	12 4	8.7	2.13	41	12.5	4 11	30	14.5	5.24
10	73	M	–	< 5	8	5.1	9 1	2.10	44	14.3	5.62	40	16.5	5.56

[1] All had tapeworm ova in their faeces before worm expulsion. Patient No. 5 had normoblastic bone mar[row]. All the others had megaloblastic bone marrow

pg/ml (the average value was 130 pg/ml) The normal average value 331.8±7.30, (9 was never reached during the period of the study

After final remission the peripheral blood picture remained normal. This, and the gradual normalization of the serum B_{12} content, must be taken as evidence that also the patients with achlorhydria suffered from fish tapeworm anaemia, and that there were no cases of genuine pernicious anaemia in this series.

The curves obtained for the individual patients (Figs. 1 and 2) were of such similar configuration that closer analysis was undertaken.

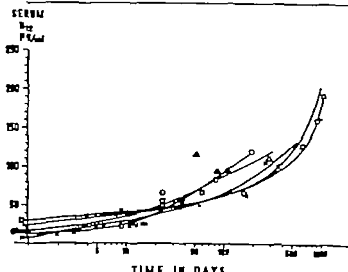


Fig 1 Graph describing the serum vitamin B_{12} concentrations as function of time in subjects N 1—5

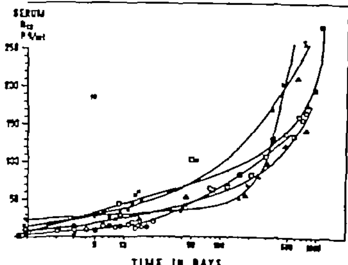


Fig 2 Graphs describing the serum vitamin B_{12} concentrations as function of time in subjects N 6—10

For the mathematical analysis the following assumptions and simplifications were made: The dietary amount of vitamin B_{12} absorbed daily was taken to be constant. Dynamic equilibrium was assumed to exist between the serum pool and the extravascular pool. Continuous incorporation and elimination of the vitamin was presumed to take place in the blood and the elimination of B_{12} was expected to change with time. Thus we did not assume *a priori* that the amount of B_{12} lost per unit time is dependent only on the amount of B_{12} present in the body. That this elimination is also a function of the time elapsed since the incorporation of the vitamin has been demonstrated by Heinrich and Pfau (6).

If we assume that the daily percentile elimination is p_1 during the first t_1 days after the incorporation of an amount a, p_2 during the following t_2 days, etc. ending at p_k after $t_1+t_2+\dots+t_{k-1}$ days, the equation describing the serum vitamin B_{12} level t days after expulsion of the tapeworm can be written

$$y_t = y_0 e^{-0.01 p_n t} + \sum_{i=1}^{n} \frac{100a}{p_i} e^{-0.01 \sum_{j=1}^{i-1} p_j t_j} (1 - e^{-0.01 p_i \tau})$$

in which

y_0 = serum vitamin B_{12} concentration before expulsion of the worm,

n = a number dependent on t and defined from the inequality

$t_1 + t_2 + \dots + t_{n-1} < t \leq t_1 + t_2 + \dots + t_n$

a = the daily amount of vitamin B_{12} incorporated in the serum,

$$\tau = \begin{cases} t_i \text{ for } i = 1, 2, \dots, n-1 \\ t - (t_1 + t_2 + \dots + t_{n-1}) \text{ for } i = n \end{cases}$$

$t_0 = 0$

If the average curve for the increasing serum B_{12} concentrations equals this equation, it must be possible to estimate empirical values for a, y_0 and the p's. In this material the initial mean value

SERUM
B_{12}
µµg/ml

300

— ESTIMATED GRAPH
--- OBSERVED GRAPH

200

100

1 10 50 100
TIME IN DAYS

Fig. 3. Mean curve obtained from the serum concentrations compared with the estimated curve.

is $y = 15$ pg/ml. If t is a very short time, then $y \approx y_0 + at$ and thus from the observed value $y_{10} = 31.7$ we find $a = 1.67$. After these values had been inserted in the equation, the following step was to seek one or more values for the p's.

If we assume a constant value for p, i.e. $p_1 = p = \ldots = p_n$, we end up with a graph which differs significantly from the curve observed experimentally. Further analysis indicates that the values for p must have decreased with time. This decrease in the p value is naturally continuous, but to obtain a good approximation of the curve observed it is sufficient to estimate three mean values for p during three successive time intervals. These mean values were

$p_1 = 3.0$ for $t \leq 50$ days,
$p = 0.7$ for $50 < t \leq 150$ days, and
$p = 0.05$ 0.005 for $150 < t$ days.

Thus if the estimated values are inserted in the formula, with an assumed p_3 value of 0.02 the following equations represent the serum B_{12} concentration curve (Fig. 3)

$t \leq 50$: $y = 15\, e^{-0.0002}$
$+ 56(1 - e^{-0.03\, t})$
$50 < t \leq 150$: $y = 15\, e^{-0.0002}$
$+ 97 - 53.5\, e^{-0.007\,(t-50)}$
$150 < t$: $y = 15\, e^{-0.0002}$
$+ 995.5 - 925\, e^{-0.0002\,(t-150)}$

Discussion

Haematological remission in tapeworm anaemia, after the removal of the worm, is rapid and can be completed within some two months, as we know from a great number of previous studies (1, 2). In this respect the present investigation revealed nothing new. It is obvious, however, that serum vitamin B_{12} concentrations are a much better criterion of any existing B_{12} deficiency than peripheral blood values alone. In tapeworm-infected patients with low serum B_{12} levels to whom no other therapy is given except expulsion of the worm, the B_{12} deficiency state may continue for months before normal serum B_{12} concentrations are attained. Approximately 6 months elapse before the "border line" between pathological and normal serum B_{12} values is reached.

From a therapeutic standpoint, the interpretation of the results is that it takes more than a year (average 400 days) before the vitamin B_{12} deficiency can be regarded as cured. Since approximately 50 per cent of all fish tapeworm carriers have low serum vitamin B_{12} concentrations, the implication of the result is that worm carriers should be given vitamin B_{12} during and after their worm cure even in cases where no anaemia is present.

The curves describing the serum B_{12} concentrations as a function of time in the patients studied were amazingly similar and formed a satisfactory basis for calculations of the vitamin B_{12} incorporation into and elimination from the serum pool. The turnover of vitamin B_{12} includes metabolic steps which are impossible to measure at present, and in calculations of the dynamics of the vitamin, crude approximations have to be made. This is of course also true of the mathematical analysis in our investigation. However, the results are in good agreement with those of earlier kinetic studies. Newman *et al.* (8) assume that vitamin B_{12} first spreads to all subcellular fractions and later on concentrates in the

mitochondria in a state where its turnover rate is very slow Whole body counting studies (6) have shown that the average daily loss of an oral dose of radiovitamin B_{12} diminishes as a function of time. This is confirmed by our results, which show that the elimination rate of vitamin B_{12}, as calculated from the serum concentrations, decreases with time. Compared with the results obtained with whole body counting however our calculations give considerably higher values for the daily loss during the first 50 days. Heinrich and Pfau (6) found a radiovitamin B_{12} elimination rate of 0.6 per cent on the 30th day Our corresponding value was 3 per cent during the first elimination period ($t \leq 50$ days) During the second and third periods, the estimated elimination rate gradually decreased from 0 7 per cent to between 0 005 and 0 05 per cent.

We have assumed that a dynamic equilibrium exists between the plasma and tissue pools and that the serum vitamin B_{12} concentration measures all vitamin B_{12} pools. On the other hand it has been suggested that the turnover of vitamin B_{12} is best described as an open system which includes one or more compartments with slow turnover rates, and that in such a system equilibrium will not be attained in a finite time (10, 11) In serum, which is considered to be the transport pool, the turnover rate is rapid. Thus, in principle no true equilibrium between serum and extravascular pools exists, but this does not significantly influence the calculations made However it is inconceivable to think of the high elimination rate found during the first 50 days as reflecting the elimination rate for the whole body One possible explanation is that, in the early stages, instead of an approximate equilibrium between the blood and extravascular pools, the tissues (i.e. the haematopoietic system) incorporate more vitamin than the plasma does. This would explain the rapid haematological remission. Later on, a state very similar to equilibrium is reached In fact, the third value calculated for p ($p_3 =$ 0 005 – 0 05) in our study was of the same order as that determined by Heinrich and Pfau (0 051 per cent)

Summary

10 patients infected with fish tapeworm received no therapy other than dosage with anthelminthics. Their mean serum vitamin B_{12} level before the worm cure was 15 pg/ml. After the expulsion of the worm, their serum vitamin B_{12} concentrations were followed for 6–36 months. Haematological remission occurred within 2 months and there were no relapses. The material offered an unusual opportunity to perform turnover calculations based on the serum vitamin B_{12} values, which continually increased from nearly zero to normal in an otherwise healthy human organism. Analysis of the graphs obtained showed a vitamin B_{12} elimination rate of 3 per cent in the first 50-day period from the beginning of vitamin B_{12} incorporation into the serum. Then the daily elimination decreased gradually to 0 7 and finally to about 0.02 per cent. The results indicate that in the first period the extravascular pools receive larger amounts of vitamin B_{12} than they do later on after haematological remission has occurred. From the clinical point of view the study

indicates that after expulsion of the worm it may take more than a year before the vitamin B_{12} deficiency is spontaneously corrected

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Renal Failure and Serum Vitamin B_{12} Concentrations

By

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The significance of unusually high serum vitamin B levels is in most instances obscure. It is known however that an elevated serum B_1 concentration can occur in some blood dyscrasias, hepatic disorders and renal failure (5) Except in very typical cases of myeloproliferative diseases, the finding of a high B content in the serum is of limited diagnostic value. The phenomenon is of some theoretical interest, however During the years 1961—1963 we have had the opportunity to follow the serum vitamin B values in patients with severe acute and chronic renal failure. The results differ in some respects from those of other workers and will therefore be reported.

Material and Method

The series studied comprised 14 patients with acute tubular necrosis and 20 patients with chronic renal failure. Because of pronounced renal insufficiency all patients in the acute group and 13 patients in the chronic group were subjected to haemodialysis. Seven subjects in the latter group had renal failure of moderate degree, and therefore no dialysis was performed. The primary causes of the acute tubular necrosis are given in Table I and the diagnoses of the patients with chronic renal disease are evident from Table II The plasma creatinine values are included in the tables in order to give an idea of the degree of renal failure. No other clinical data or results of laboratory investigations will be presented, because no correlation between them and the serum B_1 concentrations could be detected.

The control series consisted of 636 vitamin B_1 determinations performed during the years 1956—1963 on healthy persons and hospital patients not suffering from renal diseases or other conditions known to affect the serum vitamin B_1 level. These controls were collected within the regions of the cities of Vasa and Helsingfors.

The serum vitamin B_{12} was assayed microbiologically with *Euglena gracilis* strain z, according to the method of Hutner Bach and Ross (2) The vitamin B_1 level in the serum was determined before and after dialysis, and in some patients the assay was repeated 2—3 times.

Result

The microbiological vitamin B_1 activities in the investigated sera are seen in Tables I and II In both the acute and

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the chronic group of renal failure a slight increase in the serum vitamin B_{12} concentrations during haemodialysis was observed in some cases. This elevation was not significant, however. The mean serum vitamin B_{12} level in the chronic group was 596.2±54.2 pg/ml and in the acute group 529.8±88.0. The corresponding value for the controls was 351.8±7.3. Thus a highly significant difference in the serum vitamin B_{12} concentrations between the control group and the patients with acute renal failure was found ($t = 4.20$, $p < 0.001$). No significant difference was seen between the controls and the group with chronic renal insufficiency.

There was no correlation between the vitamin B_{12} content of the serum and the plasma creatinine values or any other parameter of renal insufficiency.

Table I. Serum vitamin B_{12} concentrations and plasma creatinine values before and after haemodialysis in patients with acute tubular necrosis. The diagnoses refer to the primary cause of the renal damage.

Case No.	DIAGNOSIS	Serum B_{12} pg/ml		Plasma creatinine mg/100 ml	
		Before dialysis	After dialysis	Before dialysis	After dialysis
1	Mercury chloride intoxication	488	604	18.9	7.1
		524	500	19.1	9.3
2	Premature separation of placenta.		282	15.0	7.6
	Caesarean section	446	541	20.3	7.2
		334	376	20.0	9.3
3	Crush syndrome	672	672	17.5	11.8
		984	984	19.1	9.8
4	Gall-bladder disease	644	720	13.6	8.2
5	Tumour of the left kidney. Nephrectomy	324		13.2	
6	Tumour of the right kidney	192	108	16.9	11.2
	Nephrectomy	432	420	21.3	11.3
7	Cerebral contusion. Multiple fractures	2184	1888	17.0	9.6
8	Sequels after abortion	447	512	17.0	10.2
		560	683	20.0	7.6
9	Electrocution. Burns	400	512	15.5	13.4
		420	400	13.4	14.3
		410	400	18.0	9.6
10	Premature separation of placenta	680	770	16.3	7.5
		420	400	15.9	9.3
11	Drug	[illegible]	177	14.5	8.6
12	Pulmonary infarction	400	420	16.4	10.8
13	Sequels after endarterectomy	240	170	15.5	8.1
14	Poisoning with industrial solvents	702	348	4.0	4.0
	Mean	529.8 88.0			

Discussion

Many explanations may be advanced to account for elevated serum B_{12} concentrations in renal failure. Matthews and Beckett (3) discuss, *inter alia*, the possibility of an increased serum vitamin B_{12} binding capacity, failure of utilization of vitamin B_{12} by the bone marrow, failure of excretion of the vitamin and a possible release of vitamin B_{12} from necrotizing renal tissue. As long as our knowledge of the role of the kidneys in vitamin B_{12} metabolism is incomplete we may accept them all at least as contributory factors in raising the serum vitamin B_{12} level in patients with renal failure. However we want to make some comments on this and on our own results.

The excretion of vitamin B_{12} by the kidneys is limited, the daily excretion

Table II. Serum vitamin B_{12} concentrations and plasma creatinine values before and after haemodialysis in patients with chronic renal failure.

Case No.	DIAGNOSIS	Serum B_{12} pg/ml		Plasma creatinine mg/100 ml	
		Before dialysis	After dialysis	Before dialysis	After dialysis
15	Chronic glomerulonephritis	232	240	28.0	14.0
16	Chronic pyelonephritis	540	640	10.6	6.8
17	Chronic glomerulonephritis	452	588	11.9	5.7
		444	280	14.5	6.5
18	Chronic glomerulonephritis	248	244	15.0	6.3
19	Subchronic glomerulonephritis	216	180	18.0	10.2
20	Chronic pyelonephritis	400	460	14.5	5.9
21	Subacute pyelonephritis	171	152	12.1	6.8
	Chronic interstitial nephritis	444	412	17.5	8.0
22	Subacute glomerulonephritis	540	180	11.9	5.8
		128	128	13.2	8.0
		200	130	19.7	13.0
23	Chronic glomerulonephritis	290	290	17.0	9.0
		580	1096	17.0	7.7
24	Chronic glomerulonephritis	236	200	28.0	16.0
25	Malignant hypertension	214	242	21.9	15.2
26	Chronic interstitial nephritis	348	340	18.0	9.8
27	Chronic nephritis	720	640	9.5	4.3
28	Renal tuberculosis	484		7.4	
29	Chronic pyelonephritis	305		10.4	
30	Chronic pyelonephritis	1496		4.4	
31	Renal tuberculosis	653		4.0	
32	Chronic pyelonephritis	260		2.4	
33	Chronic pyelonephritis	364		1.4	
34	Chronic pyelonephritis	155		8.0	
	Mean	396.2 $\pm$ 54.2			

amounting to 0—275 ng (1) But the main elimination route is the biliary and intestinal tract, which can probably compensate for a moderate vitamin B_{12} overload caused by a disturbed renal excretion mechanism. Such a compensatory mechanism may be insufficient, however for the elimination of an acute excess in the serum vitamin B_{12} content, for instance after parenteral administration of large amounts of B_{12} or endogenous vitamin released after tissue damage. In such cases even a slightly reduced renal excretion capacity may lead to considerably elevated serum values. This is probably what happens in acute tubular necrosis. A contributory mechanism may be an inability of the injured renal tissue to incorporate the circulating vitamin. Such damage to a possible B_{12} acceptor mechanism is in accordance with the low B_{12} contents in liver tissue observed in acute hepatitis and cirrhosis of the liver and may perhaps also explain the high serum concentrations found by Matthew and Beckett in chronic renal failure (3) In our patients with chronic renal failure the mean serum vitamin B_{12} levels did not differ significantly from those of the controls. This discrepancy between the two series is difficult to explain. The different techniques used for the B_{12} determination cannot account for the divergent results One explanation is perhaps the very great variations in the serum values seen in patients with renal failure In five persons with chronic renal insufficiency not included in this material we have found considerable variations in the serum levels from time to time in the same patient The differences are of such a magnitude that they cannot be accounted for only by the error of the assay method An extreme example is a 39-year-old male patient with renal tuberculosis, and a plasma creatinine level varying between 7 4 and 10 4 His serum vitamin B_{12} values *determined* at about 3-week intervals were as follows: 484 240 395, 124 653 416 10 000 896 1 064 2,008 pg/ml. The most obvious explanation for these variations is the variability of the disease itself with its possible influence on the intestinal vitamin B_{12} absorption, the general health and the dietary habits of the patients with varying periods of inadequate dietary vitamin B_{12} supply Rath and coworkers (4) for instance, have *shown that absorption* of radioactive vitamin B_{12} in patients with renal diseases may be even more efficient than in normal subjects. In this connexion it is also important to remember that antibiotics may cause a transient rise in the serum vitamin B_{12} concentrations. (5)

In the case of chronic renal failure the interpretation of the results is difficult and the difference between our results and those of Matthew and Beckett may be insignificant.

From the above discussion we conclude that the only type of renal failure in which a plausible explanation for the increased serum B_{12} levels may be found is acute tubular necrosis.

Summary

The serum vitamin B_{12} concentrations in 20 patients with chronic renal failure and 14 patients with acute tubular necrosis were determined and compared with those of 63 controls. The average serum vitamin B_{12} level of the acute

group was significantly higher than that of the controls. No difference in the serum vitamin B_{12} concentrations was found between the chronic group and the control group. Serum vitamin B_{12} values measured before and after haemodialysis did not differ significantly from each other. The probable cause for the raised serum vitamin B_{12} values in acute tubular necrosis is release of the vitamin from injured tissue and, possibly, damage to tissue vitamin B_{12} acceptors.

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Serum Vitamin B_{12} Levels in an Isolated Island Population

By

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In order to study the anthropological and gene characteristics in a homogeneous population, a mass investigation was performed in Kökar, an isolated island in the Baltic between Sweden and Finland. A genealogical analysis showed that the inhabitants of this island are highly endogamous (1). The examinations considered to be of interest included a study of the serum vitamin B_{12} concentration. In addition, since tapeworm infections are completely unknown in this population, it was compared with a rural population in Eastern Finland, previously examined by Nyberg *et al.* (4), which is heavily infected with the fish tapeworm, *Diphyllobothrium latum.*

Series and Methods

A field study was performed in July–August 1961 and 1962. Of the about 500 inhabitants of Kökar 221 were examined in 1961 and 300 the following year.

In 1961, 162 blood samples for vitamin B_{12} determination were drawn from 92 females and 70 males. In 1962, 255 blood samples were drawn from 140 women and 115 men. The latter figures include 87 re-examinations (35 males and 52 females).

The blood samples were collected in carefully washed centrifuge tubes. After clotting, the serum was obtained by centrifugation and transferred to Wassermann tubes cleaned in a special way (5) and then stored deep-frozen until used for vitamin B_{12} determination.

The serum vitamin B_{12} concentrations were assayed with *Euglena gracilis* strain *z* (5).

In addition, a number of haematological examinations were performed, and every subject was physically examined and questioned (for details, see ref. 1).

In this paper only the results of the vitamin B_{12} studies will be reported.

The subjects investigated were given a number and the persons performing the laboratory examinations knew neither the name of the subject nor the results of the other examinations.

The data relating to the mass investigation performed in Eastern Finland are to be found in papers by Gräsbeck *et al.* (2) and Nyberg *et al.* (5).

Results and Discussion

From the standpoint of social structure

the population of Kökar is homogeneous, with similar dietary habits, fish being the main protein source. Divergent dietary patterns such as vegetarianism were unknown among the subjects examined. No cases of fish tapeworm infection were found. Six subjects who had been gastrectomized in 1937—1960 had low serum vitamin B_{12} concentrations and were omitted from the series. No other diseases or conditions known to influence the serum vitamin B_{12} concentration could be detected in the subjects studied. The cause of the pathologically low vitamin B_{12} content in the serum (< 100 pg/ml) observed in 4 cases is therefore obscure. The fact that these subjects belonged to the same family and that other members of the family also had remarkably low serum vitamin B_{12} concentrations, may be indicative of some unknown hereditary defect. The whole family will later be more closely studied. Genuine pernicious anaemia seems to be unknown among the inhabitants of Kökar.

The results of the serum vitamin B_{12} determinations are seen in Table I and Fig. 1. A statistical analysis revealed no differences between the serum vitamin B_{12} levels noted in 1961 and 1962. The two series were therefore united. In those cases where a repeat assay was made, the mean of the two determinations was calculated.

The mean serum vitamin B_{12} level for the whole series was found to be 291.7 pg/ml. Since no statistically significant differences between the two sexes were detectable, these were treated together in the subsequent analyses. Gräsbeck *et al.* (2) have shown that the distribution of the serum vitamin B_{12} concentration in a normal population is lognormal. This has been taken into account in the analysis of the present results.

When the serum vitamin B_{12} levels were plotted against age (Table I and Fig. 1) a negative correlation was found to exist between th logarithms for the serum vitamin B_{12} values and age, the serum vitamin B_{12} concentration decreasing with increasing age. This is in contrast to the results obtained in the tapeworm district where no such correlation was found (2, 4). The studies performed in Eastern Finland included an examination of 938 control subjects without tapeworm infection. Their mean serum vitamin B_{12} concentration was 273.8 pg/ml. This value does not differ significantly from that of the present series.

Table I. Correlation between serum vitamin B_{12} level and age. The regression equation shows that the average serum vitamin B_{12} concentration begins with 345.5 pg/ml (log 345.5 = 2.5385) at the age of 0 year and decreases with 6.7 per cent per decade.

	[illegible]	Coefficient of correla[illegible]	Coefficient of regression	The constant term in the regression equation	t	P
MALES	130	[illegible]	[illegible]	2.55419	4.17	0.001
FEMALES	[illegible]	[illegible]	[illegible]	2.54014	4.[illegible]	0.001
TOTAL	[illegible]	[illegible]	[illegible]	2.53845	6.00	0.001

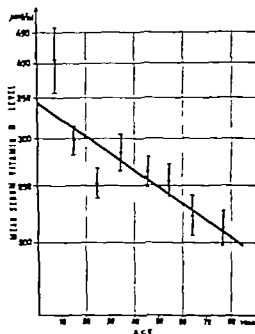


Fig 1 Mean serum vitamin B_{12} in different age groups The regression line shows the average change in the B_{12} level with increasing age

During the years 1957—1963 Nyberg (unpublished data) performed vitamin B_{12} assays on 636 serum samples from healthy subjects and hospital patients with no signs indicative of a disturbed vitamin B_{12} metabolism. The subjects in this control series were between 18 and 80 years old and came from the cities of Vasa and Helsingfors. Just as in the "healthy" series from Eastern Finland, no correlation between age and serum vitamin B_{12} concentration was detectable, but the mean serum vitamin B_{12} level was significantly higher in this urban series than in the other two series in question. The lower mean value for the Kökar population may, perhaps, be attributable to the lower standard of living of the latter as compared with Nyberg's series. The lower mean serum vitamin B_{12} level in the "tapeworm district" is probably due to the fact that the serum vitamin B_{12} level in fish tapeworm carriers rises very slowly if no treatment other than removal of the worm is given (5). Since a large proportion of this population was found to be convalescent from tapeworm infections, and since a tapeworm infection as a rule lowers the serum vitamin B_{12} concentration, it is understandable that the average serum vitamin B_{12} level was also low in the non-infected part of the population.

Summary

An endogamous isolated population was examined with regard to its serum vitamin B_{12} concentration. The serum vitamin B_{12} level was found to decrease with increasing age. The mean level was 291.7 pg/ml, which was significantly lower than the figure for a heterogeneous urban population. No cases of genuine perni

cious anaemia were detected but in one family 4 members had pathologically low serum B_{12} concentrations of obscure origin

Acknowledgement

This study was supported by grants from the Sigrid Jusélius Foundation, and the Finnish Research Council for Medical Science.

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Acta Medica Scandinavica. Suppl. 412, 1964

From the Renal Ward, Fourth Department of Medicine, University of Helsingfors, Maria Hospital, and the Folkhälsan Institute for Genetics, Helsingfors, Finland.

Plasma Creatinine in Different Sex and Age Groups of a Healthy Isolated Island Population

By

Börje Kuhlbäck, Aldur Eriksson and Henrik Forsius

Of the rough clinical measures of renal function available today the plasma creatinine concentration is the most useful, in addition to urea. It is important, therefore, to know the normal values for creatinine both in the two sexes and in different age groups, in particular. The normal values for creatinine reported during the last fifteen years are in fairly good agreement with each other (1 4 5, 8, 11 15). It has also been conclusively shown that there is a significant difference between the normal plasma creatinine values for men and women (5 10 15 19 21).

In regard of the age variations of plasma creatinine contradictory results have been reported. Josephson and Dahlberg (12) found that the creatinine concentration in the blood decreased somewhat in the higher age groups. Between 15 and 70 years no variations in plasma creatinine correlated with age have been observed. Low creatinine values have been noted in infants aged between 4—5 days and 5—6 months (11 14). In the same study the values obtained in children aged from 6 months to 6 years were of the same level as in adults. It has been observed, however that until puberty the plasma creatinine concentration is lower than in adults (23 24). The present study was undertaken in order to elucidate the normal age and sex variations in the plasma creatinine level.

Series and Methods

A total of 254 healthy subjects (115 men and 139 women) aged from 5 to 88 years were investigated on the island of Kökar in south-west Finland. Genealogic-genetical studies have shown that this population is very homogeneous (7). Its fish-rich diet differs somewhat from that of the rest of the population in this country. However diet is a factor of no influence on the plasma creatinine level (13). For practical reasons the blood samples for determination of plasma creatinine were drawn at any time of the day irrespective of what time had elapsed since the last meal. Furthermore

the urine was examined for protein and glucose and the erythrocyte sedimentation rate, the complete blood picture and the serum cholesterol level were determined. All these results were within the normal range for a mass investigation.

Plasma creatinine was determined by the Brod and Sirota (2) modification of the picrate method of Folin and W (6). True creatinine was not determined.

In addition, the plasma creatinine level was determined in 56 other Ålanders who had von Willebrand' disease (thrombopathia von Willebrand Jürgens) or belonged to so-called bleeder family. This was done since it is known that in certain conditions involving renal failure e.g. chronic glomerulonephritis, the serum sometimes contains a *heparin-like anticoagulant* which may cause haemorrhagic diathesis (3) and since it has previously been shown that capillary fragility is increased in uraemia (16). I all these cases the plasma creatinine values were normal and it seems, therefore, that the high incidence of increased capillary fragility (pathological Rumpel-Leede tests) in the Åland bleeder disease is not accounted for by nephrogenic components. Since the subjects in question in a certain sense belonged to pathological group, they were not included in the present series. In regard of age and sex differences the tendency was, however, the same in this endogamous bleeder family series as in the normal series.

Results and D scussion

(Table I Fig. 1

In the plasma creatinine there are highly significant age differences for both sexes. The significance is due to the increase up to the age of 20 after which age no significant differences occur. The ratio of the variance for adults over 20 years was for males F 5,82 0.73 and for females F 109 0.59 the plasma creatinine values were 1 106 ± 0.022 and 0. 0 ± 0.014 mg 100 ml, respectively. The differenc between the pla ma creatinine level n dult males nd females

Table I. Determinations of plasma creatinine in different sex and age groups of "healthy" isolated island population (Kökar)

Age Years	Males			Females		
	No.	Mean plasma creatinine mg/100 ml	Standard deviation	No.	Mean plasma creatinine mg/100 ml	Standard deviation
5- 9	8	.64	.07	8	.67	.02
10-14	10	79	.03	6	.86	.06
15-19	9	1.03	.05	10	.92	.04
20-29	12	1.00	.05	21	.93	.03
30-39	12	1 15	.05	22	.97	.04
40-49	18	1.06	.05	16	.98	.04
50-59	19	1 15	.04	24	.93	.03
60-69	17	1 13	.04	18	1.00	.03
70	10	1 15	11	14	.96	.05
Total	115	1.04	.02	139	.94	.01

Males F (8,106) = 8.83, P = 0.0005. Females, F (8, 130) = 4.20 P = 0.0005.

was 0 146 mg/100 ml which is highly significant (t = 6.2 201 D F P < 0 0005). These results are in good agreement with those previously reported. From the age of about 20 years, the sex difference remained fairly constant. It may be mentioned that only three women had a plasma creatinine value higher than 1.35 mg/100 ml, while nineteen men had such a value. No drop in plasma creatinine with increasing age was observable.

The plasma creatinine level in subjects aged from 5 to 20 years is of greater interest. Although the present series includes relatively few such subjects, the level was significantly lower in this age group than in adults. This is in agreement with earlier observation (Kuhlbäck, un-

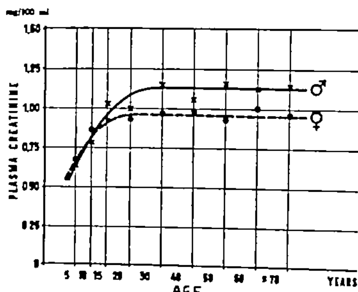


Fig 1 The plasma creatinine level at different ages in the two sexes in an endogamous island population (Kökar Åland). There are highly significant increases in plasma creatinine level in both the males (= 115) and the females (= 139) up to the age of 20. After this, the plasma creatinine level remains relatively constant in both sexes.

published) and with certain previous reports (23 24) Josephson *et al.*, however recently found that the plasma creatinine in children rose to the adult level as early as at the age of six months (14) In the same study no determinations of plasma creatinine from the ages of 6 to 18 years were made, but it must be assumed that the values, if studied would have been normal.

This discrepancy between our values and those of Josephson *et al.* is difficult to explain. As already mentioned, low plasma creatinine values have been observed in children previously too, and it has long been known that children excrete more creatine and less creatinine than adults (19 20 22) In physiological conditions, and in the majority of pathological conditions, although not in renal failure a low creatinine excretion runs parallel with a low concentration of plasma creatinine and a high creatine excretion runs parallel with a high plasma creatine level In thyrotoxicosis the ratio creatine/creatinine in the plasma and urine has been suggested as a measure of the metabolic change and the muscular damage (15) In children, high values have been obtained in the creatinine tolerance test, too (17 18) Norbye (18) stated that "eight patients who had normal creatinine value in the blood also had a definite clinical renal failure According to this it appears that a rather severe renal impairment has to be present to react on the creatinine level of the blood." — — " a normal creatinine value does not exclude the possible presence of renal failure." There is hardly any possible interpretation of these results except that the normal plasma creatinine value in children, who constituted Norbye's series (18) was lower than in adults.

Summarizing, it seems probable that the plasma creatinine level is low until puberty as compared with adults, and that the plasma creatine level is correspondingly high. To the best of our knowledge plasma creatine has not been determined in children however prob-

ably owing to the fact that all methods hitherto available have been time-consuming and unsatisfactory

When creatinine is used as a measure in the evaluation of possible renal failure, the low plasma creatinine concentration in children should be borne in mind. Values which in adults are within the upper range of the normal variation may in children be a sign of definite renal failure

Summary

The plasma creatinine level was investigated in 254 healthy subjects (115 men and 139 women) aged from 5 to 88 years. The mean value for adults ≥ 20 years of age was 1 106 ± 0 022 mg/100 ml in men and 0.960 ± 0 014 mg/100 ml in women. The difference between men and women is significant. In subjects aged from 5 to 19 years the values were significantly lower than in adults. The results and their clinical implications are discussed and co with previous observations.

Acknowledg

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Acta Medica Scandinavica. Suppl. 412, 1964

From the Renal Ward, Fourth Department of Medicine, University of Helsingfors, Maria Hospital, Helsingfors, Finland.

Serum Lipase in Acute and Chronic Nephropathies

By

A. Pasternack, B. Kuhlbäck and L. G. Tallgren

Serum lipase is an enzyme which hydrolyses glycerides of long-chain fatty acids. It is chiefly of pancreatic origin (1) and is normally excreted in the urine (11, 12).

Studies on the clinical significance of serum lipase have yielded conflicting conclusions (17). This is partly because of the inadequacy of the methods employed, partly because of the lack of a uniform procedure. It has been clearly established, however, that serum lipase is elevated in pancreatitis (6, 15). Lipase determination is of central diagnostic importance in this disease, primarily because of the relatively simple and easily reproducible method evolved by Tietz (15). Other conditions in which elevated serum lipase values have been considered possible are ileus, the use of opiates (9), parotitis (4, 16), cirrhosis of the liver (7) and biliary calculi (1).

There are scattered reports in the earlier literature of elevated serum lipase in connexion with renal disease (5, 13, 14). Gross *et al.* (9) observed serum lipase values higher than normal in only 4 out of 64 patients with azotaemia. The relationship between renal disease and serum lipase appears to have attracted little attention among research workers. Yet it is of clinical importance. In fact, upper abdominal pain is common in patients with azotaemia and elevated serum lipase may cause difficulties of interpretation in these cases.

The aim of this work was to study the relationship between elevated serum lipase and renal disease resulting in renal failure. The authors also sought to establish whether there is a difference between acute and chronic renal disease in this respect.

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When creatinine is used as a measure in the evaluation of possible renal failure, the low plasma creatinine concentration in children should be borne in mind Values which in adults are within the upper range of the normal variation, may in children be a sign of definite renal failure.

Summary

The plasma creatinine level was investigated in 254 healthy subjects (115 men and 139 women) aged from 5 to 88 years. The mean value for adults ≥ 20 years of age was 1 106 ± 0 022 mg/100 ml in men and 0 960 ± 0 014 mg/100 ml in women. The difference between men and women is significant. In subjects aged from 5 to 19 years the values were significantly lower than in adults. The results and their clinical implications are discussed and compared with previous observations.

Acknowledgement

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The aim of this work was to study the relationship between elevated serum lipase and renal disease resulting in renal failure. The authors also sought to establish whether there is difference between acute and chronic renal disease in this respect

Series

A total of 70 patients were examined. Twenty-six of them had chronic renal disease and 44 acute renal failure due to various causes. The patients were distributed among the different disease groups as shown below:

Chronic nephropathies	26
pyelonephritis	11
chronic interstitial nephritis	3
mylosdosis	3
chronic glomerulonephritis	3
hypertension	2
other	4
Acute nephropathies	44
acute tubular necrosis	34
postrenal anuria	5
acute nephritis	5

The clinical diagnosis was confirmed in most of the cases through renal biopsy and in the cases with fatal outcome at autopsy. All the cases with manifest symptoms of pancreatitis, i.e. pain and tenderness on palpation, were omitted from the study. In order to eliminate other potential disturbing factors, those patients who, in addition to an abnormally high serum lipase value also had symptoms of vomiting, ileus, cholecystopathy or liver cirrhosis or were receiving opiates were excluded from the group of acute cases. The chronic group was not selected in this way.

Methods

The serum lipase determinations were made according to Tietz (15) using a substrate of olive oil emulsion. The upper limit of normal with this method is one Tietz unit.

The plasma creatinine was determined by the method of Folin and Wu (8), modified by Brod and Sirota (3). With this method the normal values are 0.7–1.4 mg/100 ml.

The lipase and creatinine determinations were always performed simultaneously. With few exceptions the sample was taken on admission. The lipase values of some patients were followed throughout their stay in hospital.

All laboratory examinations were performed at the Laboratory Department of our hospital (Head Docent R. [illegible]).

Results

Chronic nephropathies (Fig. 1). This group comprised 5 patients whose plasma creatinine was within the normal range. Four of them had chronic pyelonephritis and clearly discernible signs of renal failure, such as slightly decreased creatinine clearance, low specific gravity of the urine and depressed phenolsulphonphthalein excretion, and 1 patient had renal amyloidosis. The creatinine values of the other patients varied, the maximum reading being 28 mg/100 ml.

Of the 26 patients of this group 11 had elevated serum lipase. The range of the values above normal was 1.06–3.56 Tietz units; no definite correlation with creatinine was established. In 7 of the 11 patients with elevated serum lipase a sudden aggravation of the condition with an oliguric episode had occurred immediately before admission, one had gall stones and jaundice and one a diffuse tenderness in the epigastrium, which might perhaps have been due to pancreatitis. The serum amylase and urinary amylase of this patient were normal however. No possibly contributing factors could be found in 2 patients.

Acute nephropathies (Fig. 2). The creatinine values varied in this group from 1.65 to 24.5 mg/100 ml. Three patients had acute glomerulonephritis, one had acute epidemic nephritis and one severe acute pyelonephritis. These 5 patients all had normal serum lipase values.

Most of the patients in the acute group, 34 in all, had typical acute tubular necrosis of varying aetiology. Five patients had postrenal anuria. It was considered justifiable to treat these 39 patients as a

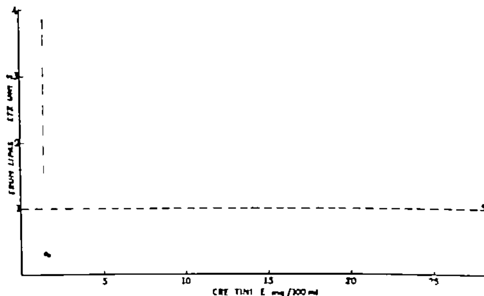


Fig 1 Correlation between serum lipase and plasma creatinine in [illegible]6 patients with chronic renal disease

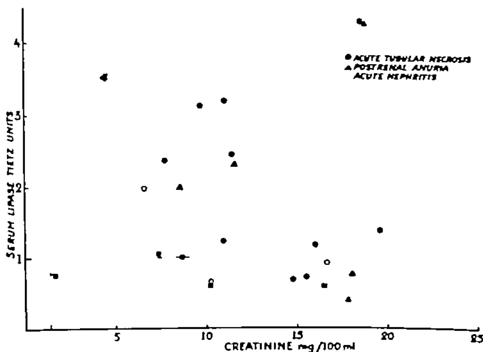


Fig 2 Correlation between serum lipase and plasma creatinine in 41 patients with acute renal disease

Series

A total of 70 patients were examined. Twenty six of them had a chronic renal disease and 44 acute renal failure due to various causes. The patients were distributed among the different disease groups as shown below:

Chronic nephropathies	26
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Most of the patients in the acute group, 34 in all, had typical acute tubular necrosis of varying aetiology. Five patients had postrenal anuria. It was considered justifiable to treat these 39 patients as a

Several patients had normal serum lipase values during the first few days of acute renal failure. On the other hand, only one normal value was obtained from the group in which the disease had already lasted over 7 days. The difference of the means of the serum lipase between the former and the latter group was statistically significant. This suggests that a fairly slow accumulation of lipase almost always occurs during acute renal failure. A point of special interest is that serum lipase values remain elevated long after the diuretic phase has been reached and the azotaemia has been corrected. It is only after the regeneration of tubular epithelium and restoration of tubular function that serum lipase tends to become normal.

It is possible that in some of the patients with chronic nephropathy changes occur in the pancreas due to uraemia and that these may be the cause of the elevated serum lipase. The increase in the lipase value and the other special features mentioned above in connexion with acute renal failure may also be attributable to pancreatitis. The absence of clinical symptoms of pancreatitis and positive autopsy findings in the series, however, do not support this theory.

It is also possible that lipase originating from renal tissue could contribute to the elevation of serum lipase in acute tubular necrosis. This mechanism requires further investigation by urinary lipase determinations and by histochemical studies in connexion with kidney biopsy.

Summary

The serum lipase of 70 patients with acute or chronic renal disease was determined. It was elevated in a total of 32 patients. The elevation of serum lipase

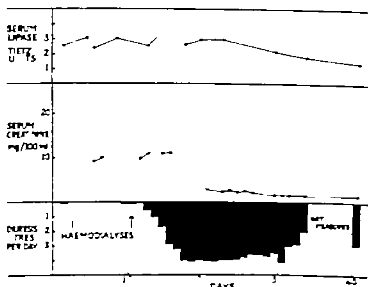


Fig. 4. Serum lipase, plasma creatinine and urinary output in typical case of acute tubular necrosis.

was associated with anuria or oliguria of acute renal failure and with the acute decompensation, accompanied by an oliguric phase, of chronic nephropathy. After its rise in these conditions the serum lipase remained elevated for rather a long time throughout the diuretic phase and after the azotaemia was corrected. Pancreatitis does not seem to play a role in the elevation of lipase at least not in acute nephropathies.

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From the Renal Ward, Fourth Department of Medicine University of Helsingfors, Maria Hospital, Helsingfors, Finland.

Serum Indican in Haemodialysis

By

A. Pasternack, B. Kuhlbäck and L. G. Tallgren

Serum indican, a non-protein nitrogen-containing substance is elevated in renal insufficiency (2 5) There are reports of the behaviour of indican in haemodialysis in the studies reported by Alwall *et al.* (1) and Kolff *et al.* (3) in which indican was observed to be dialyzable.

Material and Methods

We followed serum indican in 64 haemodialyses. The patients, 51 in all, had severe renal insufficiency of varying aetiology The serum creatinine values were 7.25–27.0 mg/100 ml and the indican values 0 57–4.25 mg/100 ml. The haemodialyses were mostly performed with the aid of Alwall' apparatus; Kolff's twin-coil apparatus was used in some instances.

The serum indican determinations were made according to the indoxyl-thymol reaction of Monias and Shapiro (4) The normal values of serum indican with this method varied from 0 05 to 0.20 mg/100 ml.

Results

Fig 1 shows the distribution of the 64 haemodialyses accord g to the percentage change in serum indican and creatinine. Indican dialyzed less efficiently than creatinine. The creatinine drop in most dialyses was over 30 per cent (mean 41 7 per cent) that of indican generally under 20 per cent. The indican values either rose or were unchanged in 13 dialyses.

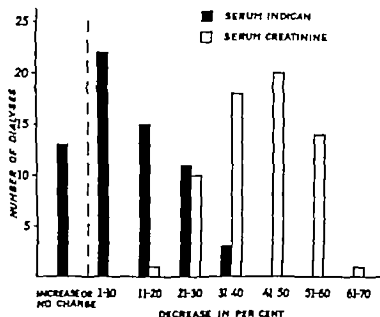
Fig 2 shows the relationship between the changes in the serum indican and creatinine values in individual dialyses. It indicates that there is a poor correlation. The change in creatinine was no smaller than usual in the 13 dialyses in which the indican level did not fall.

Fig 3 shows the mean indican values before and after dialysis. The dialyses are grouped on the basis of the percentage decrease in the indican values. The initial indican value was slightly higher on an average in the dialyses in which the fall was greater

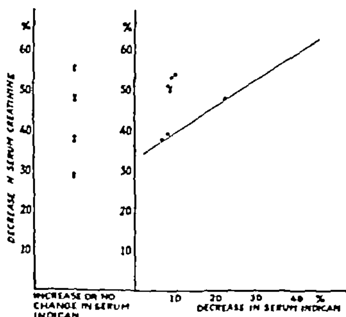
Discus ion

The following conclusions can be drawn.

1 Serum indican is dialyzable.



F g 1 Distribution [64 haemodialyses according t the pe cent g chang in serum indican (solid bars) and creatinine (pen ba).



F g 2. Relationship be tween th hange in serum creatinine and serum indican values in individual haemodialyses Th orrelat on [low degree (= +0.39) i significant (P = 0 01)

2. Serum indican is removed by dialysis considerably more slowly than serum creatinine.

3. The drop in serum indican correlates poorly with the drop in serum creatinine during dialysis.

4. The change in serum indican during dialysis is correlated with the initial value.

The slow dialysis of indican and the increase observed in some of the cases distinguishes it from other non-protein nitrogen substances. The slow dialysis may be due to the larger molecular size of indican as compared with other non-protein nitrogens. It is possible that the formation of serum indican was more rapid than its dialysis in the cases in which it rose during the treatment. Neither the clinical nor the laboratory data provided any explanation for this phenomenon. For instance, no ileus or other predisposing gastrointestinal disorder was established in this group. It is also conceivable that the increase in serum indican is caused by a reduction in plasma volume during haemodialysis. An interesting point is that the patients of this group were the most severely diseased and had the poorest prognosis.

Summary

The change of serum indican was followed in 64 haemodialyses. Indican was removed by dialysis, but much less efficiently than creatinine. In some of the cases the indican values rose or remained unchanged.

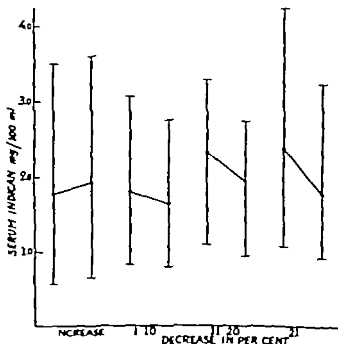


Fig. 3. Mean indican values before and after haemodialysis. The dialyses have been distributed into groups on the basis of the percentage decrease in the indican values.

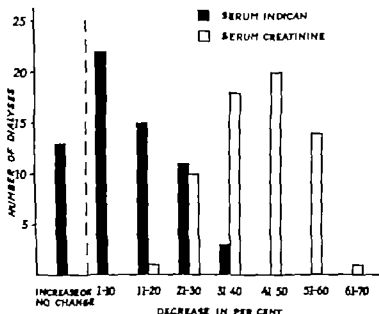


Fig 1 Distribution of 64 haemodialyses according to the percentage change in serum indican (solid bars) and creatinine (open bars).

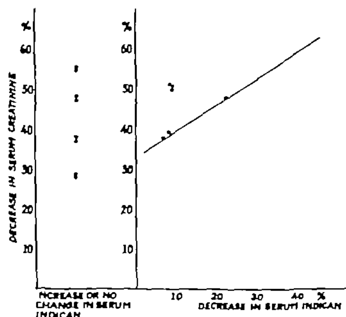


Fig 2 Relationship between the change in serum creatinine and serum indican values in individual haemodialyse The correlation of low degree (r = +0.39) is significant (P = 0.01)

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Haemodialysis in Uraemia Following Biliary Tract Surgery

By

Erik Adlercreutz and Börje Kuhlbäck

The term hepato-renal syndrome was introduced by Helwig and Schutz (4) for pathological conditions involving both the liver and the kidneys. In connexion with diseases of the liver both functional and organic renal lesions are relatively common but it is often difficult to decide whether or not a causal relationship is involved. When renal failure occurs in association with more or less acute disease of the liver or biliary tract, or after biliary tract surgery the correlation appears to be more obvious.

Circulatory renal failure has sometimes been observed in cirrhosis of the liver. According to Sheldon and Walker (10) intrinsic renal failure may in these cases result from repeated and excessive punctures for removal of ascites and frequent use of saluretics of the chlorothiazide type. A severe hepato-renal syndrome has been observed during the postoperative course after biliary tract surgery. The renal changes are mainly tubular but the terminology is somewhat confusing. Furthermore, Popper (9) reported that he had observed glomerulosclerosis in some cases of cirrhosis of the liver but this observation has not been confirmed by others.

Recently Hultén (5) pointed out that the chief pathogenic factor in the hepato-renal syndrome is shock with extremely decreased blood pressure, which affects the blood flow and causes anoxaemia. Hultén believes that a third organ, the pituitary, is involved, and owing to the resemblance between the capillary system of the latter and that of the kidneys and liver he suggests that it would be reasonable to speak of a pituitary-hepato-renal syndrome. Hultén's views concerning a pituitary involvement are very interesting.

According to the general view the prognosis is very poor in the presence of both hepatic and renal failure and it has even been stated that no therapy has any success in such cases. The death-rate varies between 65 and 100 per cent.

Shaldon and Walker however attempted haemodialysis in cases of hepatorenal syndrome associated with various diseases of the liver (10). Of 9 patients on whom a total of 20 haemodialyses were performed 4 recovered. Two patients treated conservatively died. Other authors, too, have reported favourable results with haemodialysis in cases of hepato-renal syndrome (1, 2, 6).

Series

At the Renal Ward of the Fourth Department of Medicine a relatively large number of patients with cholestatic conditions and uraemia have been treated with haemodialysis. The present series includes only cases in which cholecystectomy or choledochotomy had been performed and serious renal failure with oliguria-anuria, requiring haemodialysis, developed during the

Table I. Clinical observations in 10 cases of uraemia following biliary tract surgery

Shock	marked	6 cases
	slight	3 cases
	none	1 case
Dehydration	marked	7 cases
	slight	2 cases
	none	1 case
Ileus	marked	5 cases
	none	5 cases
Jaundice	marked	6 cases
	slight	2 cases
	none	2 cases
Oliguria		9 cases
Anuria		1 case

Table II. Postoperative complications in 10 cases of uraemia following biliary tract surgery

Gastrointestinal haemorrhages	2 cases
Biliary peritonitis	3 cases
Thromboembolism	1 case
Pancreatitis	2 cases
Pyelonephritis and other infections	6 cases

Table III. Laboratory values before and after haemodialysis in 10 cases

	J. H.		A. S.		E. S.		S. H.	
	Before HD	After HD	Before HD	After HD	Before HD	After HD	Before HD	After HD
Hb g/100 ml	11.7	12.8	9.5	11	11.1	13.2	15.0	12.0
Urea mg/100 ml	370.7	177.6	502	128	286.4	166.9	—	—
Creatinine mg/100 ml	12.25	5.95	10.6	4.9	8.80	5.20	14.3	9.2
Uric acid mg/100 ml	6.7	6.4	19.4	8.4	12.4	9.8	29.1	14.8
Total protein %	6.6	7.3	5.2	6.7	5.4	6.6	7.0	6.2
Indican mg/100 ml	1.06	1.04	1.83	1.55	0.91	0.91	—	—
K meq./l	4.2	4.2	3.0	3.4	6.0	4.2	6.4	4.7
Na meq./l	123	125	134	132	132	127	125	130
Mg meq./l	3	2.2	3.67	2.27	1.89	1.51	3.44	2.43
Ca mg/100 ml	7.9	11.6	7.6	11.5	9.6	12.5	8.6	10.9
P mg/100 ml	11.5	7.1	6.0	3.7	8.9	6.3	13.5	6.2
CO_2 vol. %	57	52	44	47	34	32	27	—
pH	7.43	—	7.46	—	7.22	7.32	7.28	—
P_{CO_2} mm Hg	40	—	36	—	32.5	35	—	50
Standard bicarb. meq./l	—	—	—	—	13.5	18.5	—	—
Base-excess meq./l	—	—	—	—	12	-6	—	—
Number of haemodialyses	1		1		1		1	
Survival	+		+		+		0	

HD = Haemodialysis

postoperative course The series consists of 10 patients, [illegible] 4 men and 6 women, operated upon either in Helsingfors or somewhere else in Finland. I 4 cases radiological examination of the bile ducts had been made during the operation. One patient was operated upon under Fluothane anaesthesia.

On admission to the Renal Ward the patients were mostly in very poor condition. The main clinical data are listed in Table I Postoperative complications were contributory cause of the poor condition of the patients (Table II)

In the majority of cases the situation was so critical that haemodialysis was performed immediately or one or two days after admission. The most important data relating to the azotaemia, the electrolyte balance, the acid-base balance and other factors of interest are listed in Table III which shows the situation both before and after dialysis.

Results

The results of therapy (including not only the effect of the haemodialyses but also the effect of all other measures taken in order to combat the hepato-renal failure) were favourable in three cases, while seven patients expired. In most cases marked jaundice and severe postoperative complications were present, which decisively contributed to the lethal outcome. The three surviving patients had been icteric, but on admission one of them had no jaundice and two were subicteric. Dehydration was present, but hypotonic shock was not observable. Thus, the situation was more favourable in these three cases than in the remainder In three of the cases with a lethal outcome a radiological examination of the bile ducts had been performed during the operation.

In five cases autopsy was performed and th histological investigation of the liver revealed more or less extensive centrilobular necroses. In no case was a

of uraemia following biliary tract surgery.

E. A.		L. L.		Ee. S.		M. J		J A.		D L.	
Before HD	After HD	Before HD	After HD	Before HD	After HD	Before HD	After HD	Before HD	After HD	Before HD	After HD
12.7	15 8	11 1	11 1	10.8	12.8	12.5	13.1	12.5	12.1	15.4	16 0
—	—	—		—	—	—	—	464 9	—	—	—
12.5	9.35	17.5	11.9	9 7	5.8	11.3	6.65	1..95	7 15	19.0	12.4
	—	22.5	17.9	18.2	9.8	17.6	10 1	18	9.2	22.9	—
6.6	6.8	—		6.5	6.5	5.5	5.5	5.2	6.6	7 4	7.8
	—	—	—	—	—	—	—	2.56	1.88	—	—
9 7	6.3	5.0	3.3	3.3	3.1	4 0	4.9	5.9	5.5	4.5	4.8
145	123	132	124	124	126	132	133	131	127	141	143
	—	2.4	1.5	1.5	1.5	2.3	2.6	3.31	2.92	3.73	2.90
7 4	7.9	10.2	9 7	9 7	11 9	7 7	12.2	8.0	14.5	9.3	9.3
10.2	10.1	—	—	8.2	5.6	4.2	4.5	6.6	4.7	3.5	3.5
46	—	23	—	6..	52	80	93	62	56	59	53
7 28		7 12	—	7 41	—	—	—	7.34	7.38	—	—
50		25	—	34	—		—	70	33	—	—
21	—	—		22		—	41	28 5	19.3	—	—
3	—	—	—	1		—	—	+8	-4	—	—
1 0		1 0		0		1 0		1 0		1 0	

Results of the first haemodialyses

lesion of the hepatic artery observable. Two patients had pancreatitis. The renal histological changes were found in the tubules (with dilated tubules, flattened epithelium, in some cases tubular necrosis, and interstitial reaction with oedema) in four cases. In the fifth case extensive cortical necrosis was observed, while the presence of tubular changes could not be established with certainty. It may be mentioned that Larsen and Thomsen found similar tubular changes in 15 cases of hepatorenal syndrome examined *post mortem* (6).

Discussion

The question as to whether it is justifiable to regard the hepato-renal syndrome as a clinical entity is still a matter of debate. Sherlock states categorically that "there is little value in retaining the term hepato-renal syndrome" (11). She believes, however, that the presence of jaundice may contribute to the development of tubular necrosis. Larsen and Thomsen (6) are adherents of Sherlock's views. By contrast, Caroli (3) who 12 years ago introduced the concept "*angiocholite urigène*" emphasizes that it is mainly cholestatic diseases of the liver which cause renal lesions, and that these develop in particular in cases which have not been operatively treated. As an example it may be mentioned that in Larsen and Thomsen's series two-thirds of the patients had cholestatic diseases of the liver.

Among the factors which play a part in the development of shock with resultant disturbances of the fluid and electrolyte balance, renal failure and uraemia, Caroli (3) emphasized infection. According to him, the critical condition is due to the release of bacterial endotoxins. Infection played an important part in our series also.

To an essential degree, the course is dependent on the severity of the liver lesion. Legrain (7) stated that in planning the therapy attention should primarily be paid to the liver. Undoubtedly the prognosis is poorer in the presence of severe disease of the liver. In our series, the hepatic lesion was slight in the three favourable cases, while it was mostly severe in the others. One patient obviously died of hepatic coma. That jaundice unaccompanied by severe shock would contribute to the development of tubular necrosis seems improbable, considering that symptoms of renal failure are rare in purely hepatocellular diseases associated with jaundice (hepatitis, in particular).

In clinical practice it is, perhaps, justifiable to speak of a hepato-renal syndrome. Both pre- and postoperatively diseases of the liver — cholestatic conditions in particular — may be associated with renal lesions which are mostly of the tubular necrosis type, but whose origin is not yet fully understood. In these cases attention should clinically be directed to both the liver and the kidneys. The immediate purpose of the therapy should be to counteract the disturbances of the fluid and electrolyte balance and to combat infection. If the symptoms of renal failure do not subside, haemodialysis with an artificial kidney should be resorted to. Even if the blood ammonia level is elevated, dialysis is indicated. The lethal outcome in seven of our cases was not solely attributable to the presence of severe, irreversible postoperative compli-

cations, but also to the fact that in some cases the patient was remitted for treatment too late.

On the basis of the present results it may be stated that attention should be directed mainly to the treatment of the liver lesion and its sequelae. If hepatic failure is threatening, the use of barbiturates, morphine etc., should be cut down. If extensive necroses of the liver are present, the detoxicating capacity of the latter and the formation of glucuronic acid and sulphuric acid are reduced. Consequently sufficient conjugation of toxic substances does not occur. Uncritical use of cortisone preparations should be avoided. In severe cases the administration of cortisone implies a strain upon the liver since the cortisone is coupled with the small amount of glucuronic acid still remaining (8).

Summary

Ten patients with uraemia following biliary tract surgery were treated by haemodialysis. Three patients recovered. In the remainder of cases, in which the outcome was fatal, severe postoperative complications and liver disease were present, and in six cases there was also infection.

From the standpoint of clinical practice it seems, perhaps, justifiable to speak of a hepato-renal syndrome. Cholestatic lesions of the liver in particular are frequently associated with renal disease, and the therapy should involve both the liver and the kidneys.

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From the Laboratory of the Surgical Departments, University Central Hospital, Helsinki, Finland.

The Value of Blood Ammonium Determination in Clinical Work

By

RUBEN GORDIN

As there is no single laboratory test yielding information about all the various functions of the liver a large number of different methods are employed in clinical practice. Many of these investigations reflect the functional state of the liver This group includes the bromsulphalein test, protein electrophoresis, the determination of certain coagulation factors, *etc.* Another group consists of more or less unspecific protein reactions. These enable the clinician to form an opinion regarding liver function, although theoretically they only reflect changes in the relative amounts of various serum proteins, *e g* the albumin-globulin ratio, which is also influenced by other factors.

Attempts at further development of the diagnostic methods relating to liver disease are still being made. Among the techniques of interest in this connexion mention may be made of liver biopsy the determination of enzymes, *e g* alkaline phosphatase, GOT GPT *etc* A method which has been hampered by technical difficulties is the determination of blood ammonium. In addition to earlier microdiffusion techniques and their modifications (1 11 16, 19 20 23) new methods have been introduced, for instance the ninhydrin method (15) In clinical investigations the correlation between laboratory results and clinical symptoms has been unsatisfactory however and this has been attributed to inadequacy of the methods. An improvement of the latter has been achieved with the introduction of ion exchange resins (4 10) which has eliminated many of the previous sources of error

As already mentioned, there is a considerable discrepancy between the blood ammonium values on the one hand, and the clinical symptoms and other laboratory tests on the other For this reason ammonium tolerance tests have been introduced, involving the determination of the ammonium level in the blood for

several hours after the patient has in gested some kind of ammonium salt (5 7 12) Egense regards these tolerance tests as more valuable than random tests, since the latter may yield normal results although the responses to tolerance tests are obviously pathological According to him, a pathological ammonium tolerance test is indicative of cirrhosis of the liver or abnormal collateral circulation, while a normal result is not obtained in the presence of severe liver disease (3) Toler ance tests have also been found useful in the evaluation of the results when a porta-caval shunt anastomosis has been performed (2, 5 6, 8, 9)

Method and Series

In this study the ion exchange method elaborat ed by Dienst was used, which during long trial period has proved to be reliable from the clinical standpoint. Only venous blood was investigated. The anticoagulant used in the tests was either heparin from The Vitamine Company New York, N Y or Heparin "Medica Since heparin preparations may contain small amounts of ammonia (3) the former were repeatedly assayed. The blood samples were examined as soon as possible after collection as a rule within one hour The results of random tests made immediately after the drawing of blood and up to 2 hours later showed no major differences, however as was also pointed out by Dienst. The normal values found by Dienst are between 0 and 0 42 μg per ml of venous blood, with mean of 0.25 μg per ml. Our mean value is somewhat lower bout 0.22 μg per ml. The method has been in clinical use since autumn 1961 at the Laboratory of the Surgical Departments, University Central Hospital, and it has also been employed in the investigation of experimental cirrhosis in dogs. The results of these studies will be published separately (25)

The clinical series consists of 21 cases of cirrhosis of the liver treated at the II Surgical Department (Head at that time: Professor Vaino Seiro, M.D) 12 patients with other diseases of the liver and 20 normal" cases in part normal subjects, in part patients with diseases not entailing meta bolic disturbances. I co-operation with the Renal Ward, Fourth Department of Medicine, Maria Hospital (Head. Professor Bertel von Bonsdorff, M.D) pilot study was made concerning the ammonium level in 15 patients with various renal diseases.

Results (Fig 1)

In all the 20 *control* cases the values fell below the limit for the pathological (0.42 μg per ml) and the majority were clustered around 0.25 μg (D)

The *liver cirrhosis* group with 21 cases was divided into two subgroups, accord-

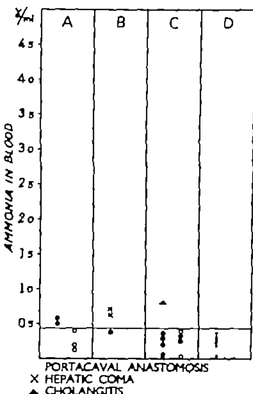


Fig 1 The ammonium level in the blood in patients with A) cirrhosis of the liver with slightly impaired liver function, B) cirrhosis of the liver with seriously impaired liver function, C) other diseases of the liver and in D) control cases

ing to whether liver function, as indicated by the tests, was fairly good (A) or highly pathological (B)

A. Of the 11 patients in this group, 6 had normal ammonium values, while in 4 of the cases with pathological values the latter were under 0.75 µg per ml. In one case the value was high, or 2 µg In this group a porta-caval anastomosis had been performed in 5 cases. This makes the group less uniform.

B This group consists of 10 patients with seriously impaired liver function tests. In one case a porta-caval anastomosis had been performed. Five patients were in a state of hepatic coma. It is striking that 6 patients had ammonium values under 0 75 µg per ml. Of these, 2 were in hepatic coma. One patient had an entirely normal value while another who was not in coma, had an ammonium value of over 3 µg per ml.

Other liver diseases (C) In this group of 10 patients, 9 had entirely normal ammonium values. On patient with severe cholangitis and cholangiolitis had an elevated value of 0.7 µg per ml (Table I)

Table I. Distribution of the cases with impaired liver function according to diagnosis.

Acute hepatitis	2 cases
Cholelithiasis and cholecystitis	3
Cholangitis	1
Thrombosis of vena lienalis	1
Malignant liver neoplasm	2
Metastasizing gastric carcinoma	1

Renal diseases A total of 15 patients with various severe renal lesions were investigated. The patients were hospitalized t the Renal Ward, Fourth Department of Medicine Maria Hospital. In 11 cases the ammonia values were within the normal range. In 3 cases the values were somewhat elevated, although under 0.6 µg and one patient had a value near 1 µg per ml.

Discussion

The poor correlation between the blood ammonium values and the severity of hepatic failure has previously been attributed to inadequacy of the methods used. The ion exchange techniques now employed have eliminated many sources of error however and must be regarded as fairly reliable. It appears that other factors must play a part. It is striking that the ammonium level in both veins and arteries shows wide variations. Sherlock, for instance, found that the hepatic vein contained a larger amount of ammonium than the other veins (5) On comparison of the ammonium level in arteries and veins, Hutchingson found that the arterial blood contained significantly higher concentrations. "The elevation of the arterial blood ammonia level was more closely correlated with abnormal mental states than was the venous level (10) The same was observed by Egense who noted a normal response to the ammonium tolerance test in venous blood, while the same test performed on arterial blood was obviously indicative of severe hepatic disturbance (5)

Investigations have been performed on the correlation between hepatic coma with mental disturbances and the content of cerebral i tracellular mmonium (21) Various changes in the blood are believed

to influence the passage of ammonium to the brain (29) Relevant studies have been performed by Schenker and Warren who in particular investigated the effect of varying temperatures on the cerebral ammonium level in mice (17 18) It has also been shown that elevation of the pH of the blood causes enhanced formation of ammonium, with increased uptake in the brain tissue (27 28) There must, however be other metabolites capable of causing hepatic coma. Among the metabolic disorders possibly concerned, disturbances in amino acid metabolism have been suggested. Interest has been centered on glutamic acid, which is capable of binding ammonia with formation of glutamine (26)

The relationship between the blood ammonium level and azotaemia has been very little discussed in the literature, and the available data are contradictory Fuld investigated 10 patients with various renal diseases. In 4 cases of acute glomerulonephritis he found elevated ammonium values, particularly high in uraemia (7) By contrast, Strehler reported normal values in 16 renal patients, some of whom were uraemic (24)

The present study revealed a pathological ammonium level in only a few of the patients with renal lesions, and in these cases the level was only slightly elevated in spite of the presence of marked azotaemia. The experience gained on this limited series constitutes evidence against the view that the severe toxic disturbances ensuing from grave renal failure are due to elevation of the blood ammonium level. It is possible that more systematic studies, for instance determination of ammonium in arterial blood in combination, perhaps, with ammonium tolerance tests, would clarify the relationship in renal disease. Further investigations are in progress and will be reported later (14)

On the basis of previous studies and personal results it appears that the determination of blood ammonium, is of relatively little diagnostic value. *By contrast,* for evaluation of the indications for portacaval anastomosis for evaluation of the post-operative course and for the decision in regard to the institution of haemodialysis this test may be of essential significance (13) This holds good in particular when acute liver diseases or chronic liver diseases in an acute phase are involved. The very favourable effect of dialysis on the elevated ammonium level was exemplified in a patient with cirrhosis of the liver the blood ammonium decreased from 0 7 to 0.2 μg per ml after dialysis and from 0.8 to 0 4 μg per ml after a re-dialysis (both were performed at the Renal Ward, Fourth Department of Medicine)

Summary

A series consisting of 21 patients with cirrhosis of the liver 10 patients with other diseases of the liver and 15 patients with severe renal failure was investigated in regard of the ammonium level in venous blood, using the ion exchange method introduced by Dienst. Among 11 patients with cirrhosis of the liver whose responses to other functional tests were slightly pathological, 4 had elevated ammonium values. Of the remaining 10 patients, whose other functional tests were highly abnormal, one had a normal ammonium

value and 6 had slightly elevated values. Two patients in hepatic coma had normal values. In one case of cirrhosis of the liver and elevated ammonium values, the latter were normalized as a result of haemodialysis.

In the group of 10 patients with other liver diseases, the ammonium level was pathological in one patient with severe cholangitis.

Of the 15 patients with chronic renal failure who were investigated, 11 had ammonium values within the normal range.

The conclusion is drawn that the diagnostic value of the determination of ammon um in the blood is relatively slight. By contrast, this test may be of significance for evaluation of the indications for porta-caval shunt, for evaluation of the postoperative course and for the decision in regard to the institution of haemodialysis.

Acknowledgement

The author is indebted to the laboratory nurses Miss Brita Helin and Miss Kyllikki Hietalahti for their capable technical assistance.

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The Syndromes of Obesity and of Delayed Growth in Adolescence

By

H. Hortling A. de la Chapelle, M. Frick and O Widholm

Obesity and delayed growth are not uncommon during adolescence. At this age such great changes are taking place in the production of the sex hormones that assays of these although important, are difficult to evaluate. Before the epiphyses close, determination of the bone age as an index of skeletal maturation is easy to perform routinely with the aid of roentgenograms of the hands and wrists according to the Atlas of Greulich and Pyle (9) This method provides a considerable degree of reliability but indicates the general hormone production only during the age range in question (18, 29)

The hormonal maturation of the adolescent is mainly brought about by the sex hormones, the growth hormone and the thyroid hormone. The production of sex hormones steadily increases from practically zero during late childhood to the normal amount in the full-grown individual. Apparently the oestrogens play a less important role in the regulation of skeletal maturation than the androgens. This is clear for instance, from the fact that there is only moderate retardation of the bone age in Turner's syndrome where oestrogen production is negligible (6, 12, and others) Little is known concerning growth hormone secretion during adolescence, despite recent studies with immunoassays and with the aid of the sulphation factor (7) The series studied are too small and th data difficult to interpret but it seems that at least some growth hormone activity is already observable from the fourth year onwards and perhaps this activity is somewhat increased during adolescence (3) Thyroid hormone production is relatively easy to evaluate and it remains practically unchanged during adolescence. Nutritional factors seem to influence bone maturation to a certain degree but perhaps have more effect on longitudinal

growth as such (1,27) On the whole, however it seems probable that the most important factor regulating skeletal maturation is the production of androgenic hormones in the testicles in the male and in the adrenal cortex in both sexes.

As obesity and delayed growth during adolescence and late childhood are in many respects opposite to each other it was considered that a combined study in these syndromes, of the skeletal maturation, the serum protein-bound iodine and the haemoglobin would probably throw light on the roles of hormonal and nutritional factors in the genesis of these syndromes.

Material and Methods

The majority of the adolescents studied attended an outpatient clinic for teenagers that has been functioning since the beginning of 1960 (10). The fifth form of mixed school, comprising 57 children with an average age of 15 years 7 months (14 1/4 – 16 1/2), served as control group.

In this study adolescents are considered obese when their weight exceeds the standard weight corresponding to the height at the individual' age by more than 15%. The standards were taken from the Broman-Dahlberg-Lichtenstein tables for Swedish children. Evidently these standards do not differ much from the corresponding values in Finnish adolescents (Bäckström-Järvinen, in preparation) The adolescent was considered short when the height was more than 5 below the height at the individual' age according the same standard tables The investigation scheme included full case history general clinical examination, peripheral blood picture erythrocyte sedimentation rate, protein and sugar in the urine, serum protein-bound iodine and basal metabolic rate, vaginal cytology with staining according to Papanicolaou, gynaecological investigation. thorough psychiatric investigation including Wartegg and TAT tests, and occasionally other investigations, such as roentgenograms of the sella turcica and electroencephalograms. The bone age was determined from the roentgenograms of the hands and wrists according to the Atlas of Greulich and Pyle (9) Two investigators estimated the bone age independently and the average was used to the nearest 1/4 year In general, there was fairly good agreement between the two investigators as to the bone age. When the difference exceeded 1 year third investigator' view was considered additionally The determination of the serum protein-bound iodine (PBI) was performed at the Stat Serum Institute by modification of Barker method. Results obtained with this method have been reported earlier by Hortling and Hiisi-Brummer (11) The haemoglobin was determined colorimetrically as oxyhaemoglobin in weak sodium carbonate. The haemoglobin values were not further compared with standard levels, since no attention was paid here to the basal haemoglobin values but only to a comparison between different groups of patients. The chromatin sex was determined from buccal smears and in some patients chromosome analyses were made from cultures of peripheral leucocytes (6)

The age of the patients in the study was 10 – 17 years. I the group of obese the average age was 13 years 11 months (10 – 16) in the group of short subjects 14 years 3 months (10 – 17) and in the control group it was 15 years 7 months (14 1/4 – 16 1/2) The number of individuals in the group of obese adolescents was 69 in the group of short patients 62 and in the control group 57

The material only included adolescents with a bone age of under 16 years.

Results[1)]

1 Comparison of bone age variations in obese short and control adolescents

The results are seen in Fig 1 In comparison with the control group the bone age was not significantly higher than the chronological age in the group of obese ($P > 0.05$) as a whole but significantly lower in the group of short adolescents

1) Exact data on every subject included in this study are available on request.

(P < 0 001) When however the subjects that also belong to the short group are omitted from the comparison, the bone age of the obese subjects is significantly higher than in the control group (P < 0 01) Four of the short patients are cases of Turner's syndrome with the karyotype 45/XO in two, 45/XO // 46/X, X-deletion mosaicism in the third and 46/XX in the fourth case. The great variation of the bone age in all three groups is apparent.

2 Serum protein-bound iodine in the obese and short adolescents and in the control group

The results are shown in Fig 2. It is somewhat surprising that th PBI values are in some cases rather high but nonetheless within the normal range among the short and the obese patients as compared with the values in the control group. The mean differences were significant in both instances (P < 0 01) The absence of correlation between PBI and height deviation among the short adolescents is shown Fig 3 The same was true w th regard to the PBI and the bone age (Fig 4) The obese adolescents were not included i this comparison.

3 Obesity

Of the 69 obese ad lescents, 5 were included in the control group also.

The influence of n tritional factors on bone age could be evaluated in this group from the correlation between the degree of overweight and th bone age deviation and to some extent from the haemoglobin alues.

a. Relation of degree of overweight t bone age

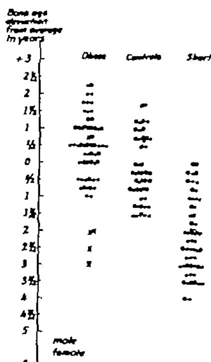
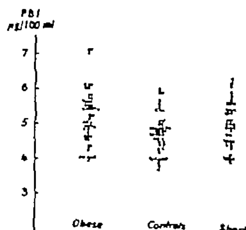


Fig 1 Deviation f bone ag from th average in th group f obese subjects, in th group f short bject and in the ontrol Th symbol × indicat ubject wh are lso included in the short group



F g 2 Serum protein-bound iodine (PBI) in the groups f obese and of hort adolescent and in th control g oup

From Fig 5 it is apparent that no correlation exists between the degree of overweight and the deviations of bone age towards earlier development.

b. Height of the obese adolescents.
In Fig 6 the height of the obese adolescents is compared with the height of the controls. The scatter seems to show that the height more often deviates towards tallness among the obese adolescents than among the youngsters in the control group The difference is not significant, however ($P > 0.05$) but when the subjects who were also short are omitted the difference is significant ($P < 0.01$) The correlation between the height deviation and the bone age deviation is shown in Fig 7 Apparently there exists a positive correlation between the degree of height deviation towards tallness and a too early skeletal maturation ($P < 0.001$)

c. Haemoglobin levels.
The haemoglobin levels for the male subjects in the obese group are compared in Fig 8 with the corresponding values for the short adolescents and the controls. Perhaps a slight tendency towards



Fig 3. Correlation between deviation from the average height and serum protein-bound iodine (PBI) in the group of short adolescents.

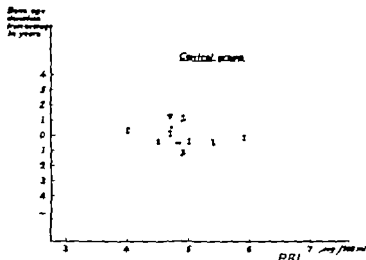


Fig 4 Correlation between deviation from the average bone age and serum protein-bound iodine (PBI) in the control group

higher values is observed among the obese patients compared with the values in the control group. The difference is not significant ($P > 0.05$) The degree of overweight is not related to the haemoglobin level (Fig 9)

4 Short adolescents Relation of height deviation to bone age Haemoglobin.

Of the 62 short adolescents, 2 were also included in the control group The correlation between the bone age and the so-called height age, *i.e* the age which, according to the standards used corresponds to a certain height is shown in Fig 10 The correlation was linear

The relation between the degree of height deviation and the bone age deviation is shown in Fig 11 From the scattergram a positive correlation is apparent between height deficit and bone age retardation ($P < 0.001$)

Some comments on Fig 11 are necessary Of the points on the −16% height deviation the 2 lowest ones, with a bone age of $-^1/_4$ year represent 2 sisters whose final height 3 years later when the epiphyses were already closed was only 138 cm. They are apparently exceptions with a genetically conditioned extreme shortness of stature. At the same height deviation of −16% the highest point at a bone age deviation of − 6 years is also interesting This girl had suffered from coeliac disease as an infant, she apparently still had intestinal malabsorption and her serum iron was low (15 μg/100 ml)

The haemoglobin level in the short adolescents examined did not differ as a group, from the level in the controls. When considering the two sexes it may be observed that the girls had haemoglobin values that were on the same level as in the control group and among the obese subjects, whereas the boys haemoglobin was lower than that of the control group ($P < 0.001$) This finding

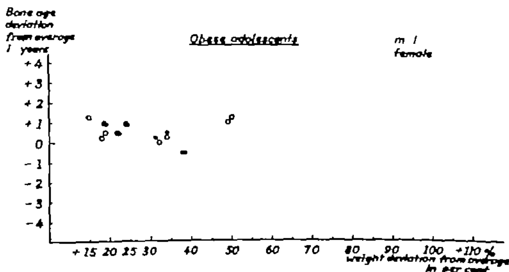


Fig 5. Correlation between the degree of overweight and the deviation from the average bone age in the obese adolescents.

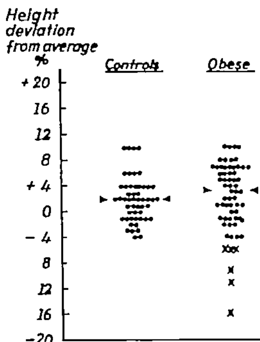


Fig 6 Deviation from the average height in the control group and in the obese adolescents. The symbol × indicates obese adolescent who are also short

will be discussed later but a different relation between the secretion of androgenic and oestrogenic hormones was probable in the short patients and the controls. The short girl with the highest haemoglobin value (12.3 g/100 ml) was a case of Turner's syndrome. The three other cases of this syndrome are not considered, as their haemoglobin was measured in another laboratory

5 *Haemoglobin. Correlation between haemoglobin levels and bone age deviation in boys and girls*

The haemoglobin levels in the obese, control and short adolescents studied are shown in Fig 8. A point worth noting is the statistically insignificant tendency towards higher Hgb values in the obese girls compared with the controls ($P > 0.05$) and the short patients. In the control group a distinct difference between boys and girls is found ($P < 0.001$)

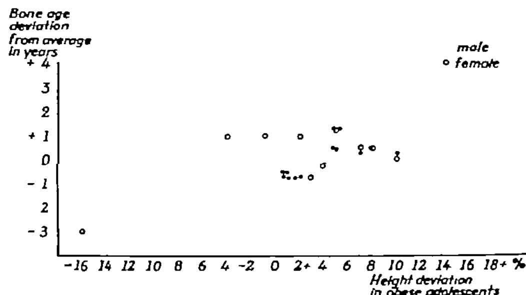


Fig 7 Correlation between the deviation from the average height and the deviation from the average bone age in the obese adolescents.

The higher age in the control group has to be remembered however and in girls it may mean a greater production of oestrogenic hormones. The short boys have clearly lower Hgb values than are seen among the boys in the control group ($P < 0.001$). The development of the secondary sex characteristics was markedly delayed in most short subjects, regardless of sex. The relation of these Hgb levels to the bone age deviations is depicted separately for boys and for girls in Figs. 12 and 13. In Fig. 12 a trend towards a positive correlation between early bone age development and higher Hgb values is seen, ($P < 0.01$) whereas no such trend is observable in the girls (Fig. 13). Rather a tendency in the opposite direction towards a negative correlation could be traced among the girls. Among the individuals with a bone age acceleration of more than 1 year in the male group 7 had Hgb values higher than 12 g/100 ml and 4 lower than 12 g and among the individuals with a bone age retardation of more than 1 year 7 had Hgb values higher than 12 g and in as many as 18 the Hgb values were lower than 12 g. The point at Hgb 7.5 g represents a girl from the control group. She was of normal height but her weight was 16% less than the average at the same age. Her erythrocyte count was 3.62 millions/mm^3 and mean Hgb per erythrocyte 21.5 pg.

Discussion

The stage during puberty before the epiphyses close affords an opportunity to study the interplay of hormonal and nutritional factors on the one hand with weight and height abnormalities on the other. During this stage the bone age is a rough indicator of the hormonal state of the organism. The androgenic hormone production is probably the most important in this respect but the

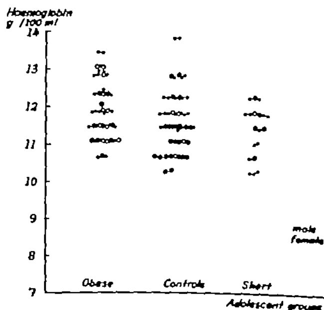


Fig. 8. Haemoglobin in the obese subject in the control group and in the group of adolescent with delayed growth.

growth hormone and the thyroid hormone as well as nutrition also influence skeletal maturation to a certain extent. The reliability of the bone age determination is, of course, difficult to evaluate, as many different factors are involved in the resulting bone age. From a practical point of view this method has attained increasing popularity as a means of assessing the general hormonal state during puberty It has proved to be valuable as a guide, when, for instance, treating extreme dwarfism with hormones (13) It has been shown that this method of estimating the bone age from the hands and wrists alone corresponds rather well with the results arrived at when the whole skeleton is considered (18)

The material in the present investiga-

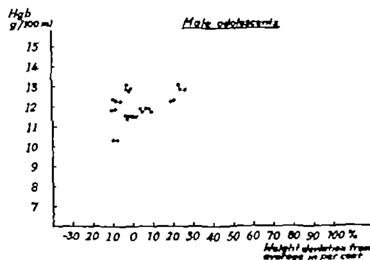


Fig 9 Correlation between the degree of overweight and the haemoglobin level (Hgb)

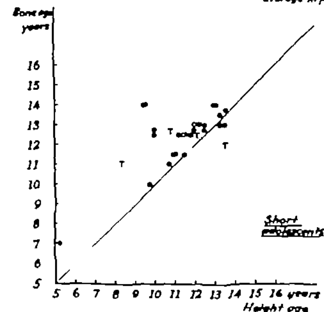


Fig 10 Correlation between bone age and height age in the short adolescents. T indicates cases of Turner's syndrome.

tion is composed almost exclusively of uncomplicated cases in which organic diseases could be excluded by careful examination. The only exceptions are 4 cases of Turner's syndrome which were diagnosed in the same series of patients otherwise considered in the study. The data on these patients are included because they did not differ much from the other short adolescents but they are marked separately in the figures. The final classification of some of the patients with overweight, delayed growth and bone age, normal sella turcica and normal thyroid function cannot be definitely decided until later in the individuals' lives. This study is thus mainly concerned with developmental deviations. The role played by the psyche is not considered here but this question will

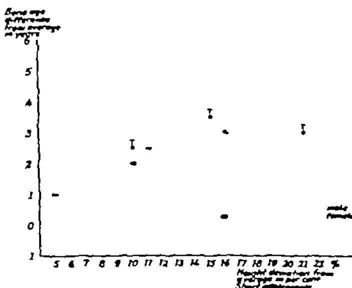


Fig 11 Correlation between the deviation from the average height and the deviation from the averag bone age in the short adolescents

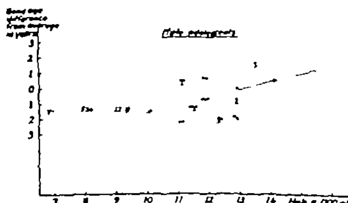


Fig 12. Correlation between the haemoglobin level and the deviation from the average bone age in the obese short and control male adolescents.

growth hormone and the thyroid hormone as well as nutrition also influence skeletal maturation to a certain extent. The reliability of the bone age determination is, of course, difficult to evaluate, as many different factors are involved in the resulting bone age. From a practical point of view this method has attained increasing popularity as a means of assessing the general hormonal state during puberty It has proved to be valuable as a guide, when, for instance, treating extreme dwarfism with hormones (13) It has been shown that this method of estimating the bone age from the hands and wrists alone corresponds rather well with the results arrived at when the whole skeleton is considered (18)

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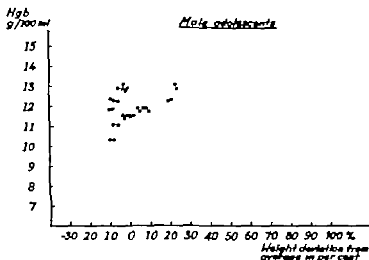


Fig 9 Correlation between the degree of overweight and the haemoglobin level (Hgb)

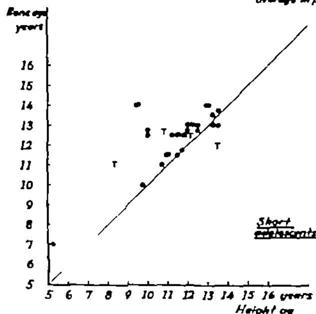


Fig 10 Correlation between bone age and height age in the short adolescents. T indicates cases of Turner's syndrome.

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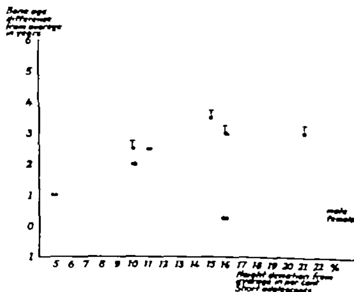


Fig 11 Correlation between the deviation from the average height and the deviation from the average bone age in the short adolescents.

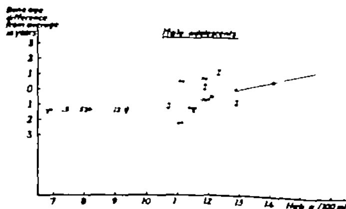


Fig 12 Correlation between the haemoglobin level and the deviation from the average bone age in the obese short and control male adolescents.

be dealt with separately (Frisk, in preparation) Nor have differences in social background been considered either

The results stress two circumstances concerning obesity namely the somewhat accelerated development of skeletal maturation and the accelerated growth as compared with the average at the same age. In this respect the observations confirm previous investigations (28 18, 26, 29) The cause of this early maturation in obese adolescents deserves some comments, however Heredity is a well known factor in the aetiology of obesity In this series overweight was also common among the relatives of the obese subjects studied. The role of nutrition was evaluated from the influence of the degree of overweight on the skeletal maturation. It was quite clearly shown by our data that the degree of overweight was *not* positively correlated with an advanced bone age. Detailed diet analyses were not carried out. This observation seems to indicate that the bone age acceleration in obesity is not secondary to the overweight but probably a primary phenomenon, perhaps with a hereditary basis.

The same thing is suggested by the observation that the growth acceleration in the obese adolescents was related to the bone age acceleration. The slight but insignificant elevation of the haemoglobin among these obese patients may well be due to other than nutritional causes.

The interpretation that there is a primary acceleration of hormonal development in obese youngsters seems to be in agreement with and to supplement some recent studies on adrenocortical function in obesity According to several authors, the cortisone turnover is increased in adult obesity the result being an increased excretion of 17-hydroxycorticosteroids in the urine but normal levels in the plasma (20 25 19 8) Migeon *et al.* (19) suggested some degree of hyperactivity in the function of the pituitary gland in obesity as was already proposed by Talbot *et al.* (28) Earlier groups of investigators, as well as Mlynaryk *et al.* (20) regarded the increased pituitary function and especially the increased cortisol production as secondary to the obesity On the other hand, Schteingart *et al.* (25) and Gogate and Prunty

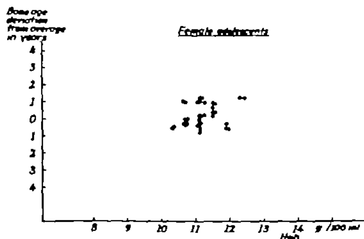


Fig 13. Correlation between the haemoglobin level and th deviation from the average bone age in the obese short and control female adolescent

(8) were unable to detect any correlation between the cortisol secretion rate and the body weight or degree of obesity Our findings support, although indirectly the opinion held by the two last named groups of investigators. Apparently an increased rate of cortisol production cannot be the only hormonal factor of importance in obesity as some acceleration of growth was also found in the adolescents with obesity whereas cortisone has been shown to inhibit growth (4 16) It appears that the role played by the growth hormone in the acceleration of skeletal maturation in obesity is still imperfectly understood. A recent report by Roth *et al.* (24) is interesting These workers measured human growth hormone in plasma by radioimmune assay and found that in markedly obese subjects an insulin induced hypoglycaemia was associated with a striking increase in human growth hormone. As insulin production is often increased in obesity (22) growth hormone production may also be increased in obesity and thus promote skeletal maturation. Nevertheless, further studies on growth hormone in adolescence and obesity are needed.

Among the obese adolescents are some (8 out f 69) whose bone age was delayed by more than 1 /₂ years. 5 of these were also short. It seems that such cases differ from uncomplicated obesity nd apparently their pituitary function is decreased. In many respects they resemble cases of adiposo-genital dystrophy but they cannot be regarded as true cases of Frölich's syndrome since their sella turcica is normal and they show no other signs of impaired pituitary or hypothalamic f nction. Probably they form a clinical entity on their own and such cases are in need of further study not least with regard to their adrenocortical function. Their sex chromatin was normal. They resemble cases described by Johnsen (14) with obesity and hypogonadism and pparently they cannot be classified as "fat boys"

In the group of adolescents who were short, the bone age was as a rule definitely delayed. The development of the secondary sex characteristics was nearly always retarded. It is probable that in those cases in which the skeletal maturation was only slightly or not at all delayed hered tary factors determined the short stature The degree of height deviation towards short stature was positively correlated with the degree of delay in skeletal maturation. This is in agreement with observations by others (29) It was not likely that weight would influence the short stature, as the weight was mostly near normal in this group and the haemoglobin of the girls did not differ from the levels in the group of obese adolescents.

Heredity influences the final height as well as the time for the growth spurt. This was also observed in the present series of youngsters. Among their relatives shortness or delayed growth was common. Hereditary shortness and delay of hormonal development are apparently dissociated and independent of each other This is illustrated by the observation of 3 sisters with a final height of 138 cm in two nd 141 cm in the third but with very slight retardation of skeletal maturation during growth. Two of them

are included in this series; the third was too old

Many authors use the term delayed puberty for uncomplicated shortness in adolescence. This is probably not quite appropriate as puberty includes other aspects besides those involved in growth. Too little is known about growth hormone production in such cases but it is certain that the production of androgenic hormones is delayed in these short youngsters. Androgenic and anabolic hormones are effective in the treatment of such delayed growth. Perhaps the term "delayed pubertal growth" would best fit our present knowledge of this type of stunted growth.

The thyroid hormone production was not related to the growth or the skeletal maturation in this series. The existence of short stature in hypothyroidism or hypopituitarism with deficiency of thyrotrophic hormone production is of course established but no such cases were included in this series of adolescents. The somewhat high values of serum protein bound iodine among the obese and short subjects were within the normal range, but nevertheless difficult to explain. The nervous tension which may exist in these groups of patients might exert some influence on the PBI values.

The haemoglobin studies were performed mainly to gain an additional impression of the significance of nutritional factors in adolescence. It was observed from the scattergrams that when boys alone were concerned a higher haemoglobin level was related to an accelerated bone age. In the control group the haemoglobin was lower among the girls than among the boys, as has previously been reported (29). These findings might depend on the amount of androgenic hormones produced and in fact a number of studies point to the possibility that the androgenic hormones may stimulate and the oestrogenic hormones depress erythropoiesis (21, 23, 15). The influence of these hormones in lower dosage is probably not very great in man as a rule. In the short boys, especially the haemoglobin was lower than in the control group or in the obese adolescents, whereas the short girls showed the same haemoglobin as was found in the other groups. The highest haemoglobin value among the short girls was observed in the only case of Turner's syndrome included in the haemoglobin series. This is interesting as in Turner's syndrome a very low oestrogen effect is found. From available reports on this syndrome it is apparent that anaemia is very uncommon (2, 30, 6, 17). It thus seems that hormonal factors have to be taken into account when the haemoglobin levels are considered in adolescence. This question needs further investigation

Summary

A study was made of 69 obese and 62 short adolescents including bone age measurements according to Greulich and Pyle, and serum protein-bound iodine and haemoglobin determinations, with the purpose of evaluating the significance of hormonal and nutritional factors in these conditions. 57 adolescents served as controls. The age of the adolescents studied ranged between 10 and $16^1/_2$ years and only individuals with a bone

age of less than 16 years were included in the series.

In the obese subjects the bone age was somewhat advanced in comparison with the controls, although a small number of obese patients showed a considerable retardation of bone age. There was also a less marked tendency to accelerated growth in this group. The degree of overweight did not influence the bone age which suggests the possibility of a primary hormonal disturbance in obesity

In the short adolescents the bone age was mostly markedly delayed The degree of height deficit was positively correlated with the delay in bone age. The same correlation between height and bone age was also found in the obese group

Serum protein-bound iodine was not correlated with the bone age or with the height of the short adolescents.

The haemoglobin level was lower among girls than among boys in the control group. Short boys but not girls, had a lower haemoglobin level than was found in the control group. The influence of sex hormones on haemoglobin levels at this age is discussed.

The terms "fat boy" and "delayed puberty" are discussed.

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A Method for the Separation and Estimation of Conjugated Oestrogens in Pregnancy Blood

By

HERMAN ADLERCREUTZ

At present there are many different methods available by which oestrogens can be chemically estimated in blood (7 8, 9 14 17 24 28 30) All these methods include hot acid hydrolysis of the conjugated oestrogens, which is known to destroy a certain amount of the steroids (1 5 16) As far as the author is aware nothing has been published concerning the chemical estimation of oestrogens in blood by a method based on enzyme hydrolysis of th conjugated oestrogens.

Recent studies (1 3 11 12, 25 29 33 34) have drawn attention to the physiological importance of oestrogen conjugation in the human organism. However comparatively little is known about oestrogen conjugation in the blood of the adult. Some evidence for the presence of both free oestrogens (13 27 29) oestrogen sulphates (29) and oest rogen glucosiduronates (11 12, 29) has been presented and Purdy *et al* (26) were able to isolate and identify oestrone sulphat in pregnancy blood nd they also obtained some quantitative results.

It therefore seemed of interest to try to develop methods for the separation and estimation of conjugated oestrogens in blood A previously published method for bile oestrogens (1) was modified for the estimation of the three classical" oestrogens in pregnancy blood. This method includes a separation of the oestrogen 3-mono-sulphates ("sulphate" fraction) from the other conjugates ("glucosiduronate" fraction) Possibly occurring double conjugates, sulpho-glucosiduronates (32, 33) are estimated in the "glucosiduronate" fraction.

The present investigation deals with the methodological problems which arose in the work with blood extracts and presents the evidence obtained concerning the reliabil'ty of the procedure developed.

Material and Methods

Blood was drawn in the morning into heparinized glass bottle from the cubital vein of hospitalized pregnant women in the last trimester measured, and immediately mixed with methanol to void hydrolysis of the unstable conjugates. Two

cord blood samples were also used in the recovery experiments.

Most of the reagents were obtained from the same sources and purified as described previously (1) except for the methanol, which was used without redistillation. Toluene, n-hexane, benzene, hydrochloric acid, dimethyl sulphate and sodium hydroxide were obtained from Merck, Darmstadt, Germany. The absolute ethanol was spectrographically pure (AaS, Oy Alkoholiliike Ab) and the nitrogen was supplied by Aga, Åbo, Finland.

The reference standards were the same as used previously (1) and the cleaning of the glassware was also described in the same study (1). It is very important to clean the Kober tubes well. After being washed with soap and water they are rinsed with distilled water and absolute ethanol.

The method for the separation and estimation of conjugated oestrogens in pregnancy blood consists of the following different steps:

1. Extraction of the 25 ml blood samples with aqueous methanol.
2. Precipitation of fatty material in cold aqueous methanol (36).
3. Separation of oestrogen "sulphates" from oestrogen "glucosiduronates".
4. Extraction of the free oestrogens from the "sulphate" fraction with ether. The free oestrogens were not analysed.
5. Enzyme hydrolysis of the conjugated oestrogens and ether extraction and washing of the ether extracts. The two fractions were processed separately.
6. A toluene-sodium hydroxide partition and ether extraction. The following steps were carried out according to Brown (4) with slight modifications according to Diczfalusy and Westman (10).
7. Separation of oestriol from oestrone and oestradiol by solvent partition.
8. Methylation.
9. Extraction of the methylated oestrogens and destruction of conta- nating su oxidation.
10. Chromatography rated a lated oestrogens.
11. Kober reaction Nocke with slight modific

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for bile oestrogens by Adlercreutz (1). Some comments are necessary especially regarding the extraction and hydrolysis of the conjugated oestrogens.

25 ml blood samples are mixed with 60 ml of methanol and extracted 4 times with 60 ml of aqueous methanol (methanol : water 70/30 v/v). The technique described by Diczfalusy and Magnusson (9) is used. The final extracts of about 300 ml re placed in deep-freeze (36) and processed as described for the bile method (1).

During the stage of separation of the "sulphate" and "glucosiduronate" fractions (stage 3) emulsions tend to be formed in work with blood samples. They do separate, however, although only after standing for some time. If the shaking of the two phases prior to the separation was done with caution the formation of emulsions could be greatly reduced. However, during later stages of the work it was found that if the precipitation of fatty material in cold aqueous methanol (stage 2) is repeated after evaporation of the extract, the subsequent tendency to form emulsions is reduced considerably. The reprecipitation of fatty material in cold methanol was carried out in a volume of 30–50 ml and no significant losses were noted. All the recovery experiments in the present study were carried out without this repeated procedure.

The hydrolysis of the conjugated oestrogens in the "sulphate" and "glucosiduronate" fractions was effected with the same *Helix pomatia* extract (Suc d'Helix Pomatia, stabilisé, standardisé, Industrie Biologique Française, Gennevilliers, France) as was used previously (1). Owing to the presence of enzyme inhibitors in the blood extracts, the "sulphate" fraction is hydrolysed with 10,000 units of phenol sulphatase per ml of reaction mixture. The incubation time is 15 h at pH 5.0 in 0.15 M acetate buffer. The amount of phenol sulphatase in the *Helix pomatia* extract has been estimated by Jarrige (13) who found 1,000,000 units per ml as tested with potassium nitrocathechol sulphate and oestrone sulphate. The amount of to be used in the incubations was calculat this figure. The "glucosiduronate" is hydrolysed with 1000 Fishman units ...idase per ml of reaction mixture in pH 4.1 for 48 h. The *Helix* 100,000 Fishman units ml.

I all other respects the method is exactly like that described for bile (1). It should be mentioned that it is necessary to carry out the spectrophotometric reading with 50 mm cells.

The gas chromatographic investigations were made with Chromalab, model 110 (Glowall Corporation, Pennsylvania, U S. A.). The preparation and use of the gas chromatographic columns were as in Horning's laboratory (35) All phases were on 100—140 mesh Gas-Chrom P in 6 ft × 4 mm coiled glass columns. The following phases were used: 1 per cent QF 1 (fluoroalkyl silicone) 1 per cent XE-60 (cyanoethyl silicone) both obtained from Applied Science Laboratories, Inc. (State College, Pennsylvania, U S. A.) and 1 per cent NGS (neopentylglycol succinat) which was obtained from General Electrics. The preparation of the trimethylsilyl ethers was made according to Luukkainen *et al.* (19 20) and the following reagents were used: hexamethyldisilazane (Fluka AG Buchs, Switzerland) trimethylchlorosilane (Fluka) tetrahydrofurane (Merck) The samples (1—2 μl) in benzene, were introduced into the gas chromatograph with Hamilton syringe.

Results

Reliability of the method

1 *Accuracy* The method was tested by adding conjugated oestrogens to the mixture of blood and methanol in amounts of 5—15 μg as free oestrogen. All the blood samples were obtained from pregnant women except two, which were cord blood. The following oestrogen conjugates were used. Oestrone-3-sulphate, 17β-oestradiol-3-sulphate, oestriol 3-sulphate, 17β-oestradiol-17-glucosiduronate and oestriol 16(17?)-glucosiduronate. In order to avoid any errors arising from a possible overlap oestriol-3-sulphate and oestriol-16(17?)-glucosiduronate were never added to the same blood sample in recovery experiments. For the same reason 17β-oestradiol 3-sulphate and 17β-oestradiol-17-glucosiduronate were never added together in these experiments. The recovery experiments were carried out as described previously (1)

The recovery of free oestrogens (oestrone, 17β-oestradiol and oestriol) added to the hydrolysed extracts was 80—90 per cent, i.e. the same as found when working with bile extracts (1) As shown previously (1) when procedures for conjugated oestrogens are tested, such recoveries do not say very much with regard to the accuracy of the whole method

The results of the recovery experiments with the oestrogen-3-sulphates are listed in Table I. It can be seen that mean values exceed 80 per cent and are in good agreement with those obtained in bile with the same compounds (1)

However difficulties arose when recovery experiments were carried out with the two preparations of glucosiduronates.

Table I Recovery of oestrogen sulphates added to the blood-methanol mixture. 5—15 μg as free oestrogen of standard added to 25 ml blood samples.

	Recovery %	Range %	No. of estimations
Oestrone 3-sulphate	82.6	74.6—89.7	10
17β-Oestradiol-3-sulphat	82.9	72.5—90.4	8
Oestriol-3-sulphate	81.2	—	1

The results of the recovery experiments varied greatly and values of 0--90 per cent were obtained, the mean value for oestriol 16(17?)-glucosiduronate (10 experiments) was 49.5 per cent and for 17β-oestradiol 17-glucosiduronate (4 experiments) 42.3 per cent. This may have been due to the presence of strong enzyme inhibitors in the "glucosiduronate" fraction of the blood samples.

However other evidence was obtained indicating that hydrolysis of the conjugated oestrogens present in the "glucosiduronate" fractions of the blood extracts took place to a satisfactory degree even in those analyses in which the recovery values were zero or almost zero for both standard preparations of glucosiduronates. If the enzyme-hydrolysed samples were extracted with ether and analysed and the amounts of oestrogens found were added to the amounts present in the "sulphate" fraction, the yields agreed well with those obtained by the method of Roy and Brown (28) for the same blood samples. The method of Roy and Brown (28) involves hot acid hydrolysis of the diluted blood samples and also estimates the free oestrogens, which are disregarded in the method presented here. Two experiments in which the two methods are compared are shown in Table II. Despite the fact that no oestriol-16(17?)-glucosiduronate or 17β-oestradiol-17-glucosiduronate was hydrolysed in the recovery experiments with the method described here, the values obtained for oestriol were higher than those obtained with the method of Roy and Brown (28) involving acid hydrolysis. Owing to the low oestradiol values no conclusions can be drawn from the results of this experiment with regard to this fraction, the amounts seem to be lower with the method presented here. The same experiment was continued by hydrolysing the aqueous phase of the extracted "glucosiduronate" fractions with acid according to Brown (4) and processing them in the same way as the enzyme hydrolysed fractions. Less than 10 per cent additional oestriol could be detected, which indicates that the oestriol conjugates present in the blood samples had been hydrolysed almost completely with the enzyme. In two additional experiments with other blood samples the "glucosiduronate" fraction was hydrolysed directly with acid but the values obtained were strikingly lower than those obtained for

Table II. Comparison of oestrogen estimation by the method of Roy and Brown (28) and that presented here on two late pregnancy blood samples.

		Method of Roy and Brown µg/100 ml	Present method µg/100 ml
	Oestrone	2.71	2.31
Case S. J.	17β-Oestradiol	0.60	0.18
	Oestriol	4.12	6.79
	Oestrone	1.60	2.30
Case C. E.	17β-Oestradiol	0.60	0.18
	Oestriol	14.13	18.29

the same blood samples with the method presented here. These results would suggest that the accuracy of the method developed is satisfactory for the "glucosiduronate" fraction also.

2. *Precision.* The precision of the method was calculated according to Snedecor (31). The values obtained for both "sulphate" and "glucosiduronate" fractions were considered together. As can be seen from Table III the results are in rather good agreement with those obtained previously in bile (1).

3. *Sensitivity.* The sensitivity of the method was calculated from the values for the precision (31). The results are listed in Table IV. Since a statistical error was previously made in the calculation of the sensitivity of the bile method, new values for this method are presented in the next article in this issue. It is seen that the sensitivity of the blood method is satisfactory and the results agree rather well with those obtained for the bile method.

4. *Specificity.* The specificity of the bile method has been tested in numerous experiments (1, 2, 18). However it seemed desirable to try to obtain at least some evidence of the specificity of the

Table III. Estimate of precision expressed as the standard deviation of the difference between two results of number of duplicate determinations (31). Values have been obtained from both the "sulphate" and "glucosiduronate" fractions of 25 ml blood samples. Amounts of oestrone and 17β-oestradiol 0–0.20 μg/25 ml blood and oestriol 0–0.40 μg/25 ml blood.

	Precision μg/25 ml	No. of duplicate estimations
Oestrone	0.020	13
17β-Oestradiol	0.021	7
Oestriol	0.044	9

Table IV. Sensitivity of the blood method. Sensitivity was calculated for single and duplicate determinations from the precision (P = 0.01 and P = 0.05). Values in μg/25 ml. Amount of oestrone and 17β-oestradiol 0–0.20 μg/25 ml blood and of oestriol 0–0.40 μg/25 ml blood.

Smallest amount distinguishable from zero

		Oestrone μg/25 ml	17β-Oestradiol μg/25 ml	Oestriol μg/25 ml
P = 0.01	Single determination	0.061	0.078	0.143
	Duplicate determination	0.043	0.055	0.101
P = 0.05	Single determination	0.044	0.051	0.100
	Duplicate determination	0.031	0.036	0.070

blood method, despite the fact that all steps involved in the procedure are essentially the same as those used in the bile method

The low oestrogen content of blood renders it very difficult to obtain sufficient amounts of oestrogen to carry out infrared absorption spectrophotometry Oertel *et al* (23) were nevertheless able to do this, but in the present investigation a new type of identification was used *viz.* gas chromatography The results obtained are shown in Table V The oestrone and oestriol fractions were fairly pure, but not entirely free from other compounds. Despite this fact the chromatograms indicated that the method is satisfactorily specific for these two compounds and the results corresponded to those found for the bile method (18) The oestradiol fraction, as in bile, presented considerable difficulties when attempts were made to obtain evidence regarding the specificity of this fraction. In bile it was impossible to isolate 17β-oestradiol definitely but some indirect evidence was found indicating that the specificity is satisfactory The results in blood show that oestradiol 3-methyl ether is present in the methylated and chromatographed fraction of the blood samples, however there are many other compounds in addition and it is not known whether they influence the Kober reaction or not. No attempts were made to identify the other peaks obtained in the gas chromatographic studies. Therefore, there seems to be a need for further investigations on the specificity of the methylated oestradiol fraction of biological samples with a low oestrogen concentration, obtained by methods based on the procedure of Brown (4)

Table V Gas chromatographic investigations of the blood oestrogens obtained with the present method. Relative retention times to cholestane of the derivatives of the reference standards and of the blood oestrogens. QF−1 = 1 per cent fluoroalkyl siloxane XE−60 = 1 per cent cyanoethyl silicone NGS = 1 per cent neopentyl glycol succinate. All phases on 100−140 mesh Gas-Chrom P 6 ft × 4 mm coiled glass columns. Pressure 2 kg/cm²

	Relative retention times					
	QF−1 170 C		XE−60 193 C		NGS 207°C	
	Blood oestrogen	Reference standard	Blood oestrogen	Reference standard	Blood oestrogen	Reference standard
Oestrone 3-methyl ether	0.63	0 63	1.62	1 62	3.76	3. 6
17-trimethylsilyl ether of 17β-oestradiol 3-methyl ether	0.56	0.56	1)	0.87	1.27	1.27
16, 17-ditrimethylsilyl ether of oestriol 3-methyl ether	—	—	1 43	1 43	1 70	1 70

1) Peak covered by another unknown peak, which could not be identified.

Specificity of the group separation of sulphates and glucosiduronates In the previous study on bile (1) observations were made indicating a rather high specificity of the procedure for the separation of oestrogen sulphates and oestrogen glucosiduronates. It was pointed out that the "sulphate" fraction contains the oestrogen 3-monosulphates, whilst the "glucosiduronate" fraction contains both the mono-glucosiduronates and possibly occurring di-glucosiduronates and/or sulpho-glucosiduronates.

It was also presumed that some oestrone 3-glucosiduronate may be extracted in the "sulphate fraction. However in blood and bile this overlap is of no essential significance, since the amount of oestrone glucosiduronate seems to be very small in comparison with the amount of oestrone sulphate, and the conclusions drawn from the investigations on blood and bile cannot be influenced even by a considerable overlap of oestrone glucosiduronate in the "sulphate" fraction That the oestrone glucosiduronate fraction is small in blood has been demonstrated by Purdy *et al.* (26) and the results obtained in the next article in this issue confirm their conclusion. The overlap of the different conjugated oestrogens in the "sulphate" and glucosiduronate" fractions is shown in Table VI It is seen that with a crude urinary extract of oestrone glucosiduronate the overlap is 46 per cent in the "sulphate" fraction 54 per cent being in this way estimated in the correct fraction The figure for the overlap is lower if the extraction of the sulphates is carried out with only two extractions instead of the three in the procedure. However in bile and blood the original procedure seems preferable, since the overlap of the other conjugates is practically negligible and the main fractions are estimated with high accuracy and

Table VI Data on the specificity of the solvent partition system used for the separation of oestrogen 3-sulphates ("sulphate" fraction) from other oestrogen conjugates ("glucosiduronate fraction) according to Adlercreutz (1) Results obtained by countercurrent distribution (K-values) and in recovery experiments on extracts of bile blood and urine

	Partition coefficient (K-value)	Per cent remaining in "sulphate" fraction	Per cent remaining in glucosiduronate fraction
Oestrone 3-sulphate	49.0	99	1
17β-Oestradiol-3-sulphate	49.0	[illegible]	~1
Oestriol-3-sulphate	13.3	97.8	2.2
Oestrone-3-glucosiduronate[1]		~ 4[illegible]	~51.0
17β-Oestradiol-17-glucosiduronate	0.02	~ 4[illegible]	~9[illegible]
Oestriol-16(17)-glucosiduronate	0.01	1	99
Oestriol-3-glucosiduronate[1]		~ 1.8	~90.2
Oestriol-3-sulpho-16(17?) – glucosiduronate[2]		1	99

[1]Crude extracts from pregnancy urine used in the [illegible]
[2]Sample obtained by the courtesy of Dr [illegible]

specificity. Some of the other data in Table VI were also obtained with crude extracts of urinary conjugates, and it can be seen that in the separation procedure for sulphates and glucosiduronates none of the naturally occurring conjugates of oestrone, 17β-oestradiol and oestriol so far isolated have an overlap exceeding 10 per cent, apart from the previously mentioned oestrone glucosiduronate.

Discussion

The methodological problems arising in the work with blood extracts were somewhat greater than those encountered with the bile extracts, especially where enzyme hydrolysis of the "glucosiduronate" fraction was concerned. In blood extracts it was difficult to achieve complete hydrolysis of added oestrogen conjugates with the glucosiduronic acid in the 16 or 17 position. As far as the oestriol conjugates are concerned, oestriol 16(17?) glucosiduronate may be a minor component in pregnancy blood and in fact we do not know which types of conjugates are present in blood. It is possible that some of the oestriol conjugates occur in the form of oestriol 3-glucosiduronate, which was first isolated by Beling (3) from late pregnancy urine. Since this compound is more readily hydrolysed than the 16(17?)-glucosiduronate of oestriol (Beling, personal communication and own observations), the presence of this type of glucosiduronate would explain the rather satisfactory results obtained with the method presented here. It is not impossible that oestriol phosphate also occurs in blood, this is indicated by the work of Oertel (22), who found evidence for the presence of 17-ketosteroids conjugated with sulphate, phosphate and lipids. Such a compound would be so polar that it would remain in the "glucosiduronate" fraction in the present method. Oestrogen phosphates are hydrolysed by the *Helix pomatia* extract, which contains acid phosphatase.

It was shown by Adlercreutz (1) that in bile considerable destruction of the oestrogens occurs during hot acid hydrolysis. It seems likely that this also occurs in blood. In fact, some results obtained with conjugated oestrogens added to diluted blood and processed according to Roy and Brown (28) show that the mean recovery was about 50 per cent, the results being very variable. This is in agreement with the finding of Roy (personal communication) that about 50 per cent of free oestrogens added to unhydrolysed blood are recovered with her method. Her results, like those reported here, indicate that the best way is to test the methods by adding naturally occurring conjugated oestrogens to the unhydrolysed extracts. The results also indicate that more work must be done before there can be any hope of achieving a recovery exceeding 50–60 per cent of *all* the conjugated oestrogens present in blood. Such a method is being developed in this laboratory. However, some preliminary estimations in pregnancy blood with this new method indicate that the total amount of oestriol is only slightly greater than that obtained with the method presented here, despite the fact that the recovery of added oestrogen mono-glucosiduronates is better, being about 80 per cent in the new procedure.

Some attempts were also made to estimate the free oestrogens, but the

results obtained did not seem reliable. The values were many times too low for satisfactory estimation and spectral characteristics of the colours following the Kober reaction indicated that the specificity of the final fractions was not good. Greene and Touchstone (13) also reported difficulties in obtaining specific fractions of free oestrone and oestradiol from pregnancy blood. If the saponification procedure of Brown *et al* (6) was included in the method in addition to the toluene-sodium hydroxide partition, most of the impurities seemed to disappear but simultaneously there was a considerable destruction of added free oestrogens. It seems, therefore that it is quite a difficult problem to estimate free oestrogens in blood, especially oestrone and oestradiol. In addition, the oestrogen sulphates may theoretically be hydrolysed within a few minutes of the drawing of the blood samples, since only small enzyme concentrations seem to be necessary for this process (1)

As shown in the bile studies (1) the oestrogen sulphates may play rather an important role in oestrogen metabolism as intermediary stages between the biologically highly active and the less active compounds It therefore seemed of considerable interest to develop a method for the separate estimation of these substances in blood also. The method presented here seems to be useful for this purpose. The accuracy was found to be good more than 80 per cent of added oestrogen sulphates were recovered from the blood extracts. The specificity of the separation procedure for "sulphates" and "glucosiduronates" has been further evaluated and the method used for bile samples was found to be, perhaps, even more specific when applied to blood extracts, owing to the small amount of oestrone glucosiduronate present in blood (26)

However if urine samples are processed by this separation method a considerable overlap of oestrone glucosiduronate is found, since this compound occurs in high concentrations in urine. It has therefore been necessary to include some additional steps in the method when used for urine samples. Such a procedure has now been adopted in our laboratory (Adlercreutz, to be published) With the aid of the three methods developed for the estimation and separation of conjugated oestrogens in bile, blood and urine, it seems possible to obtain more information regarding the metabolism of the oestrogens in the human organism in health and disease.

Summary

A method for the separation and estimation of conjugated oestrone, 17β-oestradiol and oestriol in human pregnancy blood has been developed. It is essentially based on a previously published method for bile oestrogens. The accuracy precision sensitivity and specificity of the method have been investigated and the observations indicate that in most respects the procedure for blood oestrogens is as reliable as that used for bile oestrogens. The method includes a separation of oestrogen 3-sulphates ("sulphate" fraction) from the other conjugates ("glucosiduronate" fraction) The specificity of this separation is discussed.

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A Comparison between Conjugated Oestrogens in Late Pregnancy Blood and Bile

By

Herman Adlercreutz

Previous investigations have shown that enterohepatic circulation of the oestrogens takes place in the human organism (1 5 7 11 12, 15 20 25) However comparatively little is known about the different processes involved in this circulation. Investigation of the different parts of it can be carried out following administration of oestrogens but essential evidence can also be obtained by comparing the oestrogen patterns on the two sides of the liver i.e. in blood and in bile without the administration of a perhaps unphysiological amount of oestrogens. For this purpose it is necessary to carry out the investigations in late pregnancy when the oestrogen concentrations are high but still physiological.

In the present investigation a comparison was made of the pattern of conjugated oestrogens in blood and bile in late pregnancy The three "classical" oestrogens were quantitatively estimated after separation of the oestrogen sulphates ("sulphate" fraction) from the other conjugates ("glucosiduronate" fraction) It was found that in some respects the pattern is very similar in blood and bile, whilst in others great differences exist. A preliminary report has been presented previously (2)

Material

Most patients were Rh-immunized, apparently healthy women in late pregnancy who were taken into the hospital for observation. Some normal cases hospitalized just before labour were also investigated. All women delivered within one month of the sampling, all children were born alive and all the placentas were normal on gross examination. The material was therefore regarded as normal despite the weak Rh-immunization, which has not been shown to influence the oestrogen metabolism in the mother The blood was drawn in the morning from the cubital vein of the women into heparinized glass bottle, measured, and immediately mixed with methanol. The bile samples were obtained by duodenal intubation as described previously (1).

Methods

The methods of Adlercreutz (1,3) for bile and blood oestrogens were used. The reliability of the procedures has been discussed in the same publications. However some additional data have been obtained. An error was made in the previous calculation of the sensitivity of the bile method (1) and new values for both the precision and the sensitivity of this method are presented in Table I. Some new results have been included in the calculations and these have been made with values obtained at lower levels than those used for the earlier calculations. The present data indicate that the smallest amount of the different oestrogens distinguishable from zero is slightly higher than that obtained previously, however the difference is so small that it is of no practical significance. Gas chromatographic investigations on the specificity of the bile (14) and blood (3) method have also been made. All the data obtained for these two procedures indicate that they are reliable. The method for bile oestrogens has now been used in another laboratory too, with satisfactory results (Breuer personal communication).

Results

The results obtained for the bile samples have been presented previously (1). However they are presented here again (Table II) in order to make a comparison between the blood and bile samples possible. In four cases (G. B., S. J., G. E., M. P.) both blood and bile samples were analysed. All the results obtained for the blood samples are presented in Table III.

The most striking feature is the difference in oestriol concentration in blood and in bile. There is 30 times more oestriol in bile than in whole blood which means that the concentration is about 20 times higher than in plasma assuming a haematocrit value of about 40 per cent (in non pregnant healthy women the mean value is 42.4 per cent (4)). The difference is due to the high concentration of polar oestriol conjugates in bile, presumably glucosiduronates (1,25) and sulpho-glucosiduronates (13). Another interesting fact is that the concentration of the individual sulphates in plasma assuming a haematocrit value of 40 per cent is roughly the same as in bile. There also seem to be more "glucosiduronates" of oestrone and oestradiol in bile than

Table I. Estimates of precision and sensitivity of the method for bile oestrogens (Adlercreutz, 1). Precision is expressed as the standard deviation of the difference between the results of a number of duplicate determinations and the sensitivity as calculated for a single and for a duplicate estimation from the precision (P = 0.01 and P = 0.05) according to Snedecor (22). Values have been obtained from both the "sulphate" and "glucosiduronate" fractions. The amounts of oestrone and 17β-oestradiol in the bile samples ranged from 0 to 0.15 μg/100 ml and of oestriol from 0 to 1.5 μg/100 ml of bile. Figures in parentheses indicate the number of estimations.

		Sensitivity Smallest amount distinguishable from zero			
	Precision μg/100 ml	μg/100 ml P = 0.01		μg/100 ml P = 0.05	
		single	duplic.	single	duplic.
Oestrone (22)	0.013	0.037	0.026	0.027	0.019
17β-Oestradiol (26)	0.019	0.053	0.037	0.039	0.027
Oestriol (17)	0.054	0.158	0.112	0.115	0.081

Table II. Determinations of oestrogens in the bile of 7 women in late pregnancy. Duplicate estimations with 10—20 ml bile samples were carried out in the four first cases. The other estimations were made with 20 ml bile samples. According to Adlercreutz (1)

Case	"Sulphate" fraction			"Glucosiduronate" fraction			Total concentration of		
	Oestrone μg/100 ml	17β-Oestradiol μg/100 ml	Oestriol μg/100 ml	Oestrone μg/100 ml	17β-Oestradiol μg/100 ml	Oestriol μg/100 ml	Oestrone μg/100 ml	17β-Oestradiol μg/100 ml	Oestriol μg/100 ml
G.B.	5.20	0.65	5.21	1.04	3.44	349.00	6.24	4.09	354.21
S.J	3.36	0.32	3.81	0.68	1.24	245.00	4.04	1.56	48.81
G.F	4.03	0.35	4.77	0.62	1.45	544.40	4.65	1.81	549.17
M.P	4.06	0.32	3.50	0.43	0.52	89.22	4.49	0.84	92.72
A.B.	5.11	0.26	3.71	1.82	1.36	247.07	6.93	1.62	250.78
K.S.	6.38	0.41	4.94	1.72	2.61	452.16	8.10	3.02	45 10
B.F	12.15	0.32	7.97	2.25	2.23	643.83	14.40	2.55	651.80
Mean value	5.76	0.38	4.84	1.22	1.83	367.24	6.98	2.21	372.08
Per cent of total	82.5	17.2	1.3	17.5	82.8	98.7			

Table III Determinations of conjugated oestrogens in the blood of 10 women in late pregnancy. Estimations were carried out on 25 ml blood samples, with the method of Adlercreutz (3)

Case	"Sulphate" fraction			"Glucosiduronate" fraction			Total concentration of		
	Oestrone μg/100 ml	17β-Oestradiol μg/100 ml	Oestriol μg/100 ml	Oestrone μg/100 ml	17β-Oestradiol μg/100 ml	Oestriol μg/100 ml	Oestrone μg/100 ml	17β-Oestradiol μg/100 ml	Oestriol μg/100 ml
A.M.	0.95	0.13	1.72	0.00	0.00	3.[illegible]4	0.95	0.13	4.96
V.J	5.38	0.87	2.24	0.13	0.38	6.32	5.51	1.25	8.56
I.R.	2.20	0.12	2.56	0.28	0.76	10.23	2.48	0.68	1[illegible].79
G.B.	5.42	0.12	1.54	0.20	0.12	6.52	6.62	0.24	8.06
S.J	2.21	0.00	1.21	0.10	0.18	5.58	2.31	0.18	6.79
C.E.	2.26	0.06	4.87	0.04	0.12	13.42	2.30	0.18	18.[illegible]
M.P	5.31	0.07	11.06	0.10	0.11	7.05	5.41	0.18	18.11
B.K.	1.97	0.15	0.45	0.00	0.44	10.[illegible]1	1.97	0.59	10.66
A.L.	1.58	0.21	0.00	1.04	0.51	15.15	[illegible].62	0.72	15.15
M.H.	1.98	0.30	2.26	0.16	0.00	9.22	2.14	0.30	11.48
Mean value	2.93	0.20	2.79	0.21	0.26	8.69	3.13	0.47	11.49
Per cent of total	93.3	43.5	24.3	6.7	56.5	75.6			

in plasma. When the percentage of the total (oestrone + oestradiol + oestriol) "sulphates" is calculated on the total oestrogens, however, a value of 39 per cent "sulphates" is obtained for blood and only 3 per cent for bile.

If the individual blood and bile values in the four cases (G. B., S. J., G. E., M. P.) are compared, the picture is not so clear and no definite correlations between the values can be seen in this limited material. However, it should be mentioned that in the case M. P. a remarkably low oestriol value in the "glucosiduronate" fraction of the bile runs parallel with a high oestriol value in the "sulphate" fraction of the blood. This seems to indicate that in this case there might have been some disturbance of the glucosiduronic-acid conjugating system of the liver.

Discussion

The results of the estimations of oestrone, oestradiol and oestriol in late pregnancy blood agree fairly well with those published previously (for reviews see 9, 10, 18). However, it is interesting to make some comparisons, since the slight differences found may be due to differences in methodology.

When the values for blood oestrogens obtained in late pregnancy in the present investigation are compared with the results obtained by other methods, it must be remembered that the free oestrogens are not estimated in this method. They have been extracted from the "sulphate" fraction following separation of the "sulphates" and "glucosiduronates". The figures obtained previously for free oestrogens in late pregnancy blood or plasma vary a great deal (8, 11, 23, 26) and no definite conclusions can be drawn at present. However, some evidence is available (8, 21) indicating that a considerable proportion of the oestradiol occurs in the free state. The low value obtained for conjugated oestradiol by the present method may be explained by the presence of relatively large amounts of free oestradiol in blood. If comparisons are made with the results of Roy and Mackay (19) obtained with the method of Roy and Brown (18), it is seen that the mean value obtained by these workers for oestradiol in the 40th week of pregnancy is 1.23 μg/100 ml of whole blood as compared with 0.47 μg/100 ml in the present study. If 50 per cent or more of the oestradiol is free, the results agree very well. The oestrone value in the same study (19) is somewhat higher (4.68 μg/100 ml of whole blood) than that (3.13 μg/100 ml) obtained in the present investigation. However, it was later shown by Roy (17) that the oestrone level of hospitalized patients fell by 50 per cent during the first few days of hospitalization. Since all the patients had been in the hospital for several days, and sometimes weeks, it is obvious that this phenomenon accounted for the lower value obtained in the present study. Since, moreover, no free oestrone was estimated, the value obtained even seems rather high, indicating a higher recovery of oestrone sulphate with the present method. In fact, the values obtained for the recovery of oestrone sulphate with the present method (82.6 per cent) (3) cannot be obtained with a method which includes hot acid hydrolysis (1, 6).

The amount of oestriol obtained in late pregnancy blood with the present method

is considerably higher (11 49 μg/100 ml) than that (9 16 μg/100 ml) found by Roy and Mackay (19) This is partly due to a higher recovery of oestriol sulphate in the present method. However there seem to have been strong inhibitors of β-glucuronidase activity in the blood extracts which certainly may lower the values obtained with the present method (3) On the other hand, the nature of the oestriol conjugates in the blood is not known and the oestriol conjugates which can be liberated by β-glucuronidase may not be the most important conjugates of oestriol in the blood as regards concentration (3)

If the value for oestrone sulphate obtained in late pregnancy blood with the present method (2.93 μg/100 ml of whole blood) is calculated on plasma, assuming a haematocrit value of about 40 per cent (4) the value obtained (4.89 μg/100 ml of plasma) agrees with that reported by Purdy *et al* (16) (5 μg/100 ml of plasma) The very different methods used for the estimation of these values indicate that they may be very near the true ones. When Purdy *et al.* (16) measured the distribution of radioactivity in plasma fractions following administration of C -oestradiol, they found 14 6 times more oestrone sulphate than oestrone glucosiduronate like material In the present investigation, in late pregnancy blood 14 0 times more oestrone was found in the "sulphate" fraction than in the "glucosiduronate" fraction. The good agreement between these two values is a further indication that the results obtained may be near the true ones.

During the preparation of this manuscript a study concerning the conjugation of oestriol in pregnancy plasma was presented by Touchstone *et al* (24) These workers based their investigations into the nature of the oestriol conjugates on the hydrolysis of these compounds with different types of enzymes. Oestriol liberated by Mylase P was regarded as sulphate and that liberated by glucuronidase as glucosiduronate. Oestriol which could only be liberated by a combination of both these enzymes was regarded as being a sulpho-glucosiduronate. After treatment of the extracts with these enzymes they obtained evidence indicating that complete hydrolysis of the oestriol conjugates had been accomplished, and no data indicating inhibition of the enzymes were presented. The recovery of free oestriol added to plasma was 80 per cent. The results obtained by Touchstone *et al* (24) and those of the present study agree rather well. However the former study like that of Roy and Mackay (19) presents slightly lower values for oestriol than those obtained with the present method. Touchstone *et al.* (24) found 11 4 μg of conj gated oestriol/100 ml of late pregnancy *plasma* in comparison with the value of 11 49 μg of conjugated oestriol/100 ml *whole blood* with the method used in the present investigation This means that the value is about 60 per cent of that obtained here. However excellent agreement is found with regard to the relative amount of oestriol sulphate in late pregnancy blood. Touchstone *et al.* (24) found 27 per cent oestriol sulphates as compared to 24.3 per cent oestriol in the "sulphate" fraction obtained in the present study

The results presented here seem to

throw further light on the phenomena associated with the enterohepatic circulation of the oestrogens. They are in good agreement with those found in some oestrogen excretion studies in bile presented previously (1) The main feature seems to be that when the oestrogens pass from the blood to the bile, they are concentrated in the bile mainly as polar oestriol conjugates which are estimated in the "glucosiduronate" fraction of the method. The concentration of the individual oestrogen "sulphates is roughly the same on both sides of the liver which seems to be a highly interesting result. It seems to support the view that the oestrogen sulphates are perhaps intermediates between the free oestrogens and the oestrogen precursors produced and the highly polar excretion forms. The sulphates seem to be essential transport forms and as such are biologically highly active as has been shown n the case of oestrone sulphate, for example, in the treatment of amenorrhoea. This compound especially may be of great physiological importance, a fact which has been pointed out previously by Purdy *et al.* (16) and Adlercreutz (1)

The results suggest that a 16α-hydroxylation and a conjugation with glucosiduronic acid in the liver are essential steps prior to the excretion of oestrogens in the bile *However until isolation studies* concerning the conjugated oestrogens in blood and bile have been carried out, no definite conclusions regarding the natur of the oestrogen conjugates can be drawn As far as the sulphates are concerned extensive studies have shown that the method used for the separation of oestrogen sulphates from other oestrogen conjugates is specific when used for the estimation of bile and blood oestrogens (1 3) However evidence has also been obtained indicating that some of the conjugated oestriol in bile is oestriol 16 (17?)-glucosiduronate (1)

It seems to be important to collect further data on the enterohepatic circulation of oestrogens, since a more thorough knowledge of this phenomenon may increase our understanding of the nature of the disturbance of oestrogen metabolism in liver disease.

Summary

The results of quantitative estimations of oestrone 17β-oestradiol and oestriol in the sulphate" and glucosiduronate" fractions of human late pregnancy blood and bile are presented. The concentrations of the conjugated oestrogens in whole late pregnancy blood are: oestrone 3 13 μg/100 ml 17β-oestradiol 0 47 μg/100 ml and oestriol 11 49 μg/100 ml. In late pregnancy bile the values are: oestrone 6.98 μg/100 ml, 17β-oestradiol 2.21 μg/100 ml and oestriol 372 1 μg/100 ml It is found that the concentrations of the individual oestrogen sulphates in blood as calculated on plasma are approximately the same as those found in bile. However if the total concentrations of the three classical oestrogens" are considered 39 *per cent consists of "sulphates" in the* blood and only 3 per cent in the bile This is mainly due to the fact that the bile contains about 30 times more oestriol than the blood and this oestriol occurs mainly as polar conjugates, presumably glucosiduronates and sulpho-glucosiduronates. The formation of oestriol and the conjugation of this oestrogen with glucosid-

uronic acid seem to be essential for the excretion of oestrogens in the bile.

It is suggested that the oestrogen sulphates, notably oestrone sulphate, play an important role in the intermediary metabolism and transport of the oestrogens in the human organism.

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Excretion of Oestrone and Oestriol in the Urine of Some Male Subjects with the Dubin Johnson Syndrome and Liver Cirrhosis Following Oral Administration of Oestradiol Benzoate

By

HERMAN ADLERCREUTZ AND KNUT-OLOF SCHAUMAN

During recent decades much interest has been focused on the metabolism of oestrogen in liver disease, but as yet little is known about the subject (for a review see ref. 1) The results obtained have been very contradictory and their interpretation difficult, since relatively little has been known about the normal metabolism of oestrogens in the liver

However in the last few years chemical methods for the isolation, identification and quantitation of oestrogen metabolites have begun to yield more information concerning the normal metabolism of these steroids in the liver One of the many reactions taking place in the liver is the conversion of oestradiol and oestrone to oestriol. *In vitro* experiments with human liver preparations (6 15 17 18) have indicated that the liver carries out a 16α-hydroxylation and this has been confirmed in studies on bile oestrogens (1 2, 4) The existence of an enterohepatic circulation of the oestrogens has been definitely established (1 2, 4 10 13 14 23 27 29) and it has been found that oestradiol and oestrone are converted in the liver to highly polar oestriol conjugates, which are excreted into the bile (1)

Previous investigations (5,8) have shown that following intramuscular administration of oestrone or oestradiol to normal human subjects the peak of urinary excretion of these two steroids occurs on the first day but that of oestriol on the second day On the basis of experimental evidence this delayed excretion of oestriol in the urine was suggested to be due to the time needed for the conversion of oestrone or oestradiol to oestriol (1,8) for the conjugation of oestriol and for the enterohepatic circulation of these steroids (1)

If this theory of delayed oestriol excretion in the urine following administration of oestrone or oestradiol is correct, diseases of the liver involving

disturbed biliary excretory function might give rise to an abnormal urinary oestrogen pattern following administration of one of the two oestrogens.

The present investigation tests this hypothesis and shows that in some special cases of liver disease the pattern of excretion of oestrone and oestriol in the urine differs from that seen in normal cases, following oral administration of oestradiol benzoate.

Material

Two of the patients (T. V. and T. J.) investigated had the Dubin-Johnson syndrome (Dubin-Sprinz syndrome: chronic idiopathic jaundice) (16). They were brothers and a full account of the clinical and histological observations made on these two patients has been presented previously by Lehtinen and Fortelius (19). There seems to have been no doubt as to the correctness of the diagnosis. The most important laboratory data relating to these cases are presented in Table I.

The other two patients had portal cirrhosis of the liver and portal hypertension. One of them (Case N. O.) was tested twice and the other patient (Case T. H.) was tested after a porta-caval shunt operation. The case histories of these two subjects were as follows:

Case N. O. A 52-year-old man with portal cirrhosis of the liver of unknown aetiology (alcoholic ?) and portal hypertension. Both the liver and spleen were enlarged and owing to hypersplenism he had a pancytopenic blood picture. Splenoportography indicated that the portal hypertension was severe. Twice he had bleedings from enlarged oesophageal veins. He had large vascular spiders and palmar erythema but no gynaecomastia. Laboratory data obtained on several occasions are shown in Table I.

Case T. H. A 50-year-old man with alcoholic cirrhosis of the liver and severe portal hypertension indicated by enlarged veins on the abdominal wall and varicose oesophageal veins. He also had ascites and oedema of the scrotum. The diagnosis was confirmed by laparoscopy. The oestrogen test was carried out 3 months after porta-caval shunt operation. The laboratory data are shown in Table I.

The controls consisted of three patients with peptic ulcer (M. V. 50 years, J. E. 45 years and L. H. 54 years), one patient recovering from an acute infection (K. B. 35 years) and a healthy man (A. B. 27 years). The liver function tests in these cases were all normal.

Methods

Twenty-four-hour urinary specimens were collected from the patients and hydrolysed or stored in the deep-freeze until processed. None of the patients was receiving meprobamate, laxatives or other drugs which have been found to interfere with the estimation of oestrogens by the method of Brown (7, 9). First one or two 24-hour samples were collected in order to determine the basal excretion values of the oestrogens and then 2 mg oestradiol benzoate (Organon) in oil was administered in milk in the morning. The oral route was chosen because in this way the absorbed oestrogens had to pass the liver except in the patient H. T. who had a porta-caval anastomosis, and perhaps also not in patient N. O., who had portal hypertension. The urine was then collected for 3–5 consecutive days.

For the estimation of the oestrogens the same reagents were used as previously (1, 3). All organic solvents were redistilled and the same reference compounds were used as previously (1).

The oestrogens in the urine were estimated by the method of Brown *et al.* (9) as modified by Diczfalusy and Westman (11). The saponification step is included after the ether extraction of the hydrolysed oestrogens as used in Diczfalusy's laboratory. The accuracy of the method has been tested with conjugated oestrogens added to urine samples prior to hydrolysis (see reference No. 1, pp. 97–98). The Kober reaction was carried out according to Nocke (22). In some experiments a toluene-sodium hydroxide partition was included in the method between the ether extraction and the saponification step as used previously (1). This step does not change the recovery of added oestrogens by the method to any significant degree and was only tried in an attempt to obtain more specific final extracts. These experiments have been described previously (3) and it was also shown that

Table I Laboratory data on the pathological series. Dubin-Johnson syndrome (*T V* and *T J*) liver cirrhosis (*T H* and *N O*)

Case	Age	Date (month and year)	Electrophoresis of serum proteins: Tot. g%	alb. %	α_1 %	α_2 %	β %	γ %	S-GOT[1]	Prothrombin %	Cholesterol, mg/100 ml	Stolte reaction[2]	Alkaline phosphatase Bessey Lowry units	Bilirubin, mg/100 ml	Meulengracht Icterus Index	BSR[3] %
T V	51	3–60	7 7	46.3	5 6	11 1	13.0	24 0	25	90	207	1 46	1 45	2.5	1 18	14
		4–60	oestrogen test													
		4–60	8 6	40.6	4.9	13.0	15 6	26.0	21	—	240	1 76	1.25	—	1 14	—
T J	60	1–61	7.6	44.3	3.8	13.7	16.5	21 7	42	92	202	1.57	1.08	—	1:20	20
		2–61	oestrogen test													
		2–61	7 4	48.9	3.0	13.5	13 2	19.4	—	—	—	—	—	4 6	—	18
T.H.	49	9–59	6.2	32.0	8.5	9.5	11.5	38.4	—	33	131	0.78	2.9	—	1:5	17
		11–59	porta-caval anastomosis													
		2–60	6.1	26.9	10.0	11.2	15.1	36.8	—	45	121	1.00	2.3	—	1 15	—
		2–60	oestrogen test													
		2–60	[illegible]	27.0	9.0	11 7	12.6	39.8	—	35	128	1.02	2.5	—	1:9	24.3
N.O.	52	6–59	7.9	37 7	3.9	9.5	13.3	33.6	—	66	243	1 17	1.5	1.58	—	—
		7–59	oestrogen test I						—	32	—	—	—	1.0	—	—
		2–60	6.2	39 8	4.2	6.3	10.6	39.1	—	48	218	0.94	1 4	—	1 10	22.14
		2–60	oestrogen test II													
		3–60	7.2	29.7	8.4	12.4	14.9	34 6	—	56	194	0.91	2.6	—	—	—

[1] Serum glutamic-oxaloacetic acid transaminase, Karmen units.

[2] Modified Takata reaction. Normal value above 1 4

[3] Bromsulphalein retention.

the method of Brown *et al.* (9) with the modifications used does not permit the estimation of oestradiol in bile-stained urine (5) owing t the presence of chromogens interfering with the final estimation of the Kober colour Therefore no alues for oestradiol will be given in the present study

Results

The results of the oestrogen tests are shown in Tables II and III It can be seen that they vary a great deal in both the pathological and the control series. However some interesting findings may be noted The values for the basal excretion are very similar in both series and no exceptionally high or low values can be noted in the pathological series. With regard to the basal excretion of oestrone and oestriol it can be concluded that in these special cases with disturbed excretory function of the liver there are no changes in the basal excretion values.

The total amounts of oestrone and of oestriol recovered from the urine during the three first days (the mean values for the control days have been subtracted from the excretion values following administration of the oestrogen) varied in both the control and pathological series. In only one of the pathological cases (T H.) can the excretion pattern with

Table II Excretion of oestrone (OE_1) and oestriol (OE_3) in the urine of 5 control subjects following oral administration of 2 mg oestradiol benzoate All values in µg/24 hours.

Case	Age	Control days mean values		1st test day		2nd test day		3rd test day		4th test day		5th test day	
		OE_1	OE_3	OE_1	OE_3	OE_1	OE_3	OE_1	OE_3	OE_1	OE_3	OE_1	OE_3
A.B.	27	4.3	3.8	149.9	18.9	25.8	37 4	16.2	35.9	8.2	21.2	–	–
L.H.	31	7.3	3.5	114.8	16.4	88.2	18.9	10.4	8.6	6.1	2.9	9.6	1.9
M.A	50	0.0	5.8	75.6	44.2	20.0	42.4	8.9	31 7	2.4	19 4	1.2	17.6
J.E.	45	2.8	3.2	108.8	13.2	19.1	20.8	11.0	19.8	5 0	8.4	3.1	3 1
B.K.	27	2.1	5.1	126.7	39.4	43.1	61.8	15 0	17 4	4 7	8.0	2.6	6.3
Mean value		3.3	4 7	115.2	76.4	39.2	36.3	12.3	22.7	5.3	12.0	–	–

Table III. Excretion of oestrone (OE_1) and oestriol (OE_3) in the urine of 2 patients with the Dubin-Johnson syndrome (T A and T J) and two patients with portal cirrhosis of the liver (N.O and T.H) following oral administration of 2 mg oestradiol benzoate All values in µg/24 hours.

Case	Age	Control days mean values		1st test day		2nd test day		3rd test day		4th test day		5th test day	
		OE_1	OE_3	OE_1	OE_3	OE_1	OE_3	OE_1	OE_3	OE_1	OE_3	OE_1	OE_3
T A	51	1 4	8 3	207 7	42.2	16.2	14.9	3.6	13.2	–	–	–	–
T J	60	3 6	4.6	139.2	96.1	3.1	14 1	2.5	10.8	2.8	9 4	1 7	7.8
N O	5.	2.5	6 6	139 0	39.3	56.1	–	17.0	23.7	4.3	5.8	2.8	3.3
		7 0	1	140 0	60 7	23.1	39.0	0.7	6.2	0.0	2.6	–	–
T H	49	1 9	2	229 7	14.5	13.3	2.3	12.5	3.3	–	–	–	

regard to the oestrone-oestriol ratio be regarded as definitely differing from the normal (Table IV) In this case there seems to have been a disturbance of the ability of the liver to carry out 16α-hydroxylation. The percentage recovery of the dose administered (as oestrone and oestriol) is also shown in Table IV There is no difference in the percentage recovery between the control and pathological series and the figures obtained agree well with those reported by Brown (7) for normal subjects following intramuscular administration of oestrone or oestradiol.

However in some respects there were general differences in the excretion of oestrone and oestriol between the controls and the pathological cases. The peak excretion of oestriol always occurs on the first day in the pathological series and in 4 of the 5 control cases it occurred on the second day The fifth control subject had only a slightly lower value for oestriol on the second day as compared with the first day which seems mainly [illegible] to the fact that the amount [illegible] the second day was 4–500 ml less [illegible] on the other five days. It has [illegible] been shown that a variation in the [illegible] of urine excreted has a marked [illegible] on the oestrogen excretion values [illegible] 1962 personal communication)

In addition, a difference in the [illegible] of oestrone is found. The [illegible] oestrone excreted on the first [illegible] compared with that excreted [illegible] second day is higher in the path[illegible] series than in the control group.

That in these specially selecte[illegible] of liver disease with disturbed [illegible] function of the liver the peak [illegible] of oestriol in the urine follo[illegible] administration of oestradiol [illegible] occurs on the first day and [illegible] more oestrone is excreted [illegible] day seem to be the [illegible] findings in the present [illegible]

Table IV Total excretion of oestrone and oestriol in the urine of 5 normal subjects and 4 [illegible] during the first 3 days following [illegible] administration of 2 mg oestradiol benzoate [illegible] (C) Basal excretion values subtracted from the values [illegible]

Case	Oestrone μg	Oestriol μg	Oestrone + Oestriol μg	[illegible]
A.B.	178.4	80.8	259.2	
L.H.	191.5	33.4	224.9	
M.V.	104.5	100.9	205.4	
J.E.	130.5	38.2	168.7	
B.K.	178.5	103.3	281.8	
T.V. (D-J)	223.3	45.4	268.7	
T.J. (D-J)	135.6	107.2	242.8	
Y.O. (C)	174.6	81.4	256.0	
T.H. (C)	231.8	13.5	[illegible]65.3	

Mean values of two tests.

Discussion

The theory that the delayed oestriol excretion following administration of oestrone or oestradiol is due to the time needed for the conversion of these oestrogens to oestriol (1 8) for the conjugation of oestriol and for the enterohepatic circulation of the oestrogens (1) is supported especially regarding the last point, by the present investigation. It is believed that in the Dubin-Johnson syndrome the liver is capable of conjugating bilirubin but unable to excrete it into the bile (16) It may therefore be presumed that in the Dubin-Johnson syndrome there is simultaneously a disturbance of oestrogen excretion into the bile, since practically all bile oestrogens are conjugated (1 27 29) and to a high degree as glucosiduronates (1,29) and sulpho-glucosiduronates (?0) Since the oestrogens are concentrated in the bile mainly as highly polar oestriol conjugates (1) the excretion pattern of oestriol in the urine must be most sensitive to changes in the excretion of oestrogens into the bile. In fact, the most striking difference between the first and second-day excretion of oestriol is found in the Dubin Johnson cases, as compared with the control series. The first-day peak of oestriol is high, the second-day excretion being only about 1/5th of that on the first day This would suggest that the delayed oestriol excretion into the urine following oral administration of oestradiol benzoate cannot be due solely to the time needed for the conversion of oestradiol to oestriol or for conjugation two liver functions which are not disturbed in the Dubin Johnson syndrome, but mainly to the excretion of oestrogens notably oestriol into the bile and the enterohepatic circulation (1) This is further supported by the results obtained for oestrone The excretion of oestrone is more rapid in the pathological series than in the control group. Since the conversion of oestradiol to oestrone cannot be responsible for the slight delay of oestrone excretion in the control group this finding indicates that the delay is caused by the enterohepatic circulation of oestradiol and oestrone (1) which seems to be impaired in the pathological series.

In one of these two Dubin-Johnson cases (T V) a cholecystectomy had previously been done. It is not known in what way this operation may have changed the enterohepatic circulation of substances. The time factor may be changed since the bile concentration period is normally rather long and during this period the oestrogens remain (?) in the gall-bladder and do not circulate. Investigations on this problem may perhaps be of value when the time needed for the enterohepatic circulation of the oestrogens is measured

It can be seen that the same phenomena occur in the two other patients. In both, the bromsulphalein retention was pathological indicating disturbed excretory function of the liver In the patient N O there seems to have been no disturbance of the liver function with regard to the conversion of oestradiol to oestriol. Since there was slight jaundice and some impairment of the liver function the glucosiduronate conjugation capacity may have been diminished to some extent in this case. However the time needed for glucosiduronate conjugation of oestrogens in the liver cannot alone be responsible for the delayed oestriol excretion in the

urine following administration of oestradiol, since it seems not to exceed about 6—8 hours in any of the experiments carried out previously (1) and usually it seems to be much shorter (1). An impaired glucosiduronate conjugation may also to a high degree influence the excretion of oestriol in the bile, since the conjugation of oestriol, with formation of highly polar substances, presumably glucosiduronates or sulpho-glucosiduronates, seems to play an important part in the excretion of this oestrogen into the bile. Therefore it is inferred that in this case (N.O.) too the occurrence of the oestriol peak on the first day is mainly due to impaired enterohepatic circulation of the oestrogens.

The last case (T. H.) is interesting since the patient had the most severely impaired liver function and the results indicate that the conversion of oestradiol to oestriol was affected, the total excretion of oestrone and oestriol being the same as in the control cases. He also had the highest value for bromsulphalein retention and the enterohepatic circulation was also disturbed as a result of the portacaval anastomosis. In this case all the factors involved in the phenomenon of delayed oestriol excretion in the urine following administration of oestradiol may have been disturbed. In fact, the peak of oestriol excretion, which was small occurred on the first day and there was a very high oestrone excretion on the first day of the experiment. The results obtained lend further support to the theory that the enterohepatic circulation of oestrogens, and especially of oestriol, is the main cause for the delayed oestriol excretion in the urine after administration of oestradiol to normal human subjects.

The use of oestradiol benzoate, a conjugated oestrogen, in the present study may be open to criticism. However the conjugated oestrogens are better absorbed than the free ones (29) and there is no report in the literature to suggest that orally administered oestradiol benzoate is metabolized in a fundamentally different way from oestradiol. The good agreement of the control values with the results obtained by Brown (8) and by Bauld *et al.* (5) indicate that this substance is metabolized as oestradiol.

The phenomena described in the present study have been investigated in order to obtain further information on oestrogen metabolism in liver disease. However these are only some aspects of the large problem. It may also be pointed out that previous investigations (1) have indicated that the conversion of oestradiol to oestriol, the conjugation of the oestrogens and their excretion into the bile are phenomena so closely related that a disturbance of one of these procedures may influence the others. In addition to the factors already discussed, another important point deserves mention. Several workers have reported delayed clearance of administered oestrogens from the blood of patients with liver disease following administration of both labelled and unlabelled oestrogens (21, 24, 25, 26, 28, 30). The study of Pincus *et al.* (24) is a very painstaking investigation with a biological oestrogen estimation method and shows the difficulties involved in the interpretation of the results obtained with such tests in liver disease. There seems to be no doubt, however that clearance

Discussion

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The phenomena described in the present study have been investigated in order to obtain further information on oestrogen metabolism in liver disease. However, these are only some aspects of the large problem. It may also be pointed out that previous investigations (1) have indicated that the conversion of oestradiol to oestriol, the conjugation of the oestrogens and their excretion into the bile are phenomena so closely related that a disturbance of one of these procedures may influence the others. In addition to the factors already discussed, another important point deserves mention. Several workers have reported delayed clearance of administered oestrogens from the blood of patients with liver disease following administration of both labelled and unlabelled oestrogens (21, 24, 25, 26, 28, 30). The study of Pincus *et al.* (24) is a very painstaking investigation with a biological oestrogen estimation method and shows the difficulties involved in the interpretation of the results obtained with such tests in liver disease. There seems to be no doubt, however, that clearance

of oestrogens from the blood is delayed in many cases of liver disease, but, as pointed out by Pincus *et al.* (29) the results obtained cannot always be related to the clinical symptoms. This is due to our imperfect understanding of oestrogen metabolism. The use of radioactive tracers in clearance studies (28) seems to be of no advantage: even less information can be obtained than with the previously used biological methods, since the estimates of total radioactivity alone do not give any information regarding the biological activity and chemical nature of the substances estimated. The very rapid transformation of the oestrogen moiety in the human organism has been demonstrated many times, especially the conversion of oestradiol to oestrone, which occurs very rapidly in many different tissues. Much more information can be obtained when biological methods are used as shown by Pincus *et al.* (24) and in the future the use of reliable chemical methods will clarify many points which cannot be satisfactorily explained at present.

Summary

The excretion of oestrone and oestriol in the urine of four male subjects with liver disease was investigated following oral administration of 2 mg of oestradiol benzoate. Two of the patients had the Dubin-Johnson syndrome, which is said to be due to impaired biliary excretion of conjugated bilirubin, and the two other cases with portal cirrhosis also showed symptoms indicating that the biliary excretory function was disturbed. Five control subjects were also investigated. It was found that the peak excretion of oestriol in the pathological series occurred on the first day and in the control series on the second day. In addition the excretion of oestrone occurred more rapidly in the patients with liver disease than in the control group. These phenomena are believed to be due to impaired enterohepatic circulation of the oestrogens in the cases with disturbed excretory function of the liver. Some problems regarding the metabolism of oestrogens in liver disease are discussed. In the Dubin-Johnson syndrome there may be an impaired excretion of oestrogens into the bile, as is the case with bilirubin. The rate of conversion of oestradiol to oestrone and oestriol seems to be normal in these patients. In one patient with severe liver cirrhosis the conversion of oestradiol to oestriol was impaired.

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Urinary Excretion of Calcium and 17 Ketogenic Steroids in Hyperthyroidism

By

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The regulatory influence of the anterior lobe of the pituitary gland and the hypothalamus on thyroid function, as well as the importance of pituitary factors in the development of exophthalmic goitre, has been well documented (7 8 18, 30). The interrelationship between the corticotropic and exophthalmos-producing activity of the anterior pituitary has been demonstrated, for instance by the recent observation of an exophthalmos-producing factor in the serum and pituitary of patients with Cushing's syndrome and acromegaly which was shown to disappear after hypophysectomy or pituitary irradiation (26). Such observations would lead one to expect signs of pituitary-adrenal hyperactivity in hyperthyroid patients and especially in exophthalmic goitre.

Early studies on adrenocortical function in hyperthyroidism as determined by the secretion of steroid substances in the urine have in general failed to demonstrate any hyperactivity of the pituitary-adrenal system. Reducing corticoids were found to be increased, however in a few cases of hyperthyroidism by Shadaksharappa *et al.* (28) and by Talbot *et al.* (29) in 1951 and Corvilain (6) noted a significant fall in the urinary 17-ketosteroid excretion of 10 hyperthyroid patients after treatment with thiouracil. In a more extensive study Selenkow *et al.* (27) reported high normal values for urinary 17 hydroxycorticoid excretion in hyperthyroidism and plasma 17-hydroxycorticoids within normal limits. In a previous study by one of the present authors (14) the urinary excretion of 17 hydroxycorticoids and 17 ketogenic steroids was studied in a group of 32 hyperthyroid patients and the mean values were found to be very significantly increased. Plasma 17-hydroxycorticoids, on the other hand showed lower values and a lower response to ACTH in hyperthyroid patients than in normal subjects. No correlation was noted between adrenocortical function,

as determined by the urinary excretion and plasma levels of corticoids, and the occurrence of exophthalmos.

Studies on the disappearance rate of infused cortisol from the plasma (21, 25) together with studies on the metabolism of adrenocortical steroids in liver disease (9) seem to suggest that the observed deviations from normal in the urinary excretion and plasma levels of adrenal corticoids in hyperthyroid patients may be attributed to increased utilization of adrenal hormones in these patients, probably due to increased levels of enzyme activity in the liver and to an increased demand of the peripheral tissues for adrenocortical steroids.

Studies on the metabolism of cortisol in hyperthyroidism performed at this hospital and at the First Department of Medicine, University of Helsinki are consistent with this view (15).

The metopirone (Su-4885) test was originally introduced into clinical practice by Liddle and his co-workers (20) and has since then proved to be a useful method for the clinical estimation of pituitary ACTH capacity (13, 11). It was thought that additional information on the pituitary-adrenal function in hyperthyroidism might be obtained with the aid of this test, and the response to orally administered metopirone was therefore investigated in a group of hyperthyroid patients with special regard to the occurrence of pituitary ACTH activity in patients with exophthalmic goitre.

Hypercalciuria is known to occur frequently in hyperthyroidism (1, 5, 16) and attributed to the increased metabolism associated with this condition, whereas hypercalcaemia is rare (10, 16, 17). Although an increased excretion of calcium in experimental animals has not been reported to respond to hypophysectomy, hypercalciuria and occasionally hypercalcaemia are known to exist in clinical conditions associated with adrenocortical hyperfunction and can be provoked by long-term administration of adrenocortical steroids which occasionally (especially in postmenopausal women) lead to marked osteoporosis and spontaneous fractures.

Estimations of 24-hour calcium excretion and determinations of plasma calcium levels were accordingly carried out during the course of the present investigation in order to find out whether any correlation existed in hyperthyroid patients between derangement of calcium metabolism and alterations of adrenocortical function.

Material and Methods

Twenty patients with hyperthyroidism and nodular goitre were examined. Sixteen were women and four men, their ages ranging from 18 to 76 years. In 11 of these cases there was exophthalmos with oedema of the eyelids (*Syndroma thyreohypophysarium*, STH) (18, 19).

In these patients the pituitary ACTH reserve was determined by means of the metopirone test during the hyperthyroid state and the same test was repeated when the patients, following adequate therapy, had been euthyroid for at least one year. The metopirone tests were performed as follows: The mean value for the excretion of urinary 17-ketogenic steroids (17-KGS) determined by the method of Appleby *et al.* (1) during two successive days was taken as the basal value. Thereafter the patients received 500 mg metopirone orally every 4 hrs for 24 hours and the excretion of urinary 17-KGS was estimated during that day and the two subsequent days. The highest daily excretion of urinary 17-KGS was regarded as the response to the metopirone test and these values are shown in Table I.

During the same time determinations of urinary calcium were carried out. Prior to these determinations the patients had been kept on standard diet low in calcium. The mean value for the urinary excretion of calcium on three successive days is also shown in Table I.

When the above-mentioned basal determinations had been carried out, the hyperthyroid patients were treated either by subtotal thyroidectomy or with 131I When the patients had been euthyroid for at least one year the metopirone tests and the determinations of urinary calcium were repeated in the same way as previously

Results

The results of the tests are shown in Table I. It can be seen that the excretion of urinary 17 KGS in the untreated hyperthyroid patients was uniformly high (average value 19.3 mg/24 hrs) and in only three instances were normal values in the range of 9.2 to 12.7 mg/24 hrs noted. The elevation of the urinary excretion of 17 KGS in these patients following administration of metopyrone was as a rule very moderate (average value 8.9 mg/24 hrs) and in only two cases was a normal response of 26.5 mg and 45 mg/24 hrs respectively

The hyperthyroid patients were divided into three different groups according to the degree of exophthalmos and the severity of the hyperthyroid state as

Table I. The results of the metopirone tests and the urinary excretion of calcium in patients with hyperthyroidism and in the euthyroid state of the same subjects.

Case No.	Age	Degree of		Metopirone test (17-KGS mg/24 hrs)						Urinary calcium mg/24hrs	
				Hyperthyroidism			Euthyroidism			Hyperthyr	Euthyr
		TT	STH	Basal	Resp.	Diff.	Basal	Resp.	Diff.		
1	18	3	2	24.0	31.1	7.1	14.0	52.0	38.0	340	17*
2	21	3	0	27.0	27.2	0.2	16.0	39.0	23.0	412	94
6	39	3	1	14.9	25.1	10.2	8.0	16.5	8.5	412	113
13	53	3	0	21.7	48.2	26.5	11.5	48.1	36.6	272	34
15	55	3	2	10.2	17.0	26.8	18.8	16.9	8.1	392	34
16	59	3	2	13.8	34.0	20.2	11.2	37.5	26.3	300	95
3	29	2	1	16.2	16.3	0.1	7.4	25.4	18.0	112	41
5	37	2	0	20.1	29.8	9.7	13.5	46.5	33.0	300	130
7	41	2	0	30.0	30.0	0.0	16.8	33.5	16.7	773	63
8	41	2	0	31.7	48.0	16.3	13.8	38.8	25.0	665	112
9	43	2	1	20.5	24.5	4.0	13.2	32.2	19.0	190	191
10	48	2	0	14.1	20.1	6.0	11.0	24.2	13.2	79	140
11	52	2	3	19.2	30.2	19.6	9.6	38.0	28.4	263	90
12	53	2	0	12.7	13.7	1.0	13.7	27.0	13.3	274	78
17	60	2	1	25.0	38.0	13.0	14.5	38.6	24.0	371	48
19	64	2	0	18.8	63.8	45.0	7.0	48.0	41.0	210	91
20	76	2	0	9.2	11.8	2.6	4.6	16.4	11.8	53	57
4	36	1	1	21.4	29.4	8.0	14.1	26.2	12.1	284	72
14	55	1	0	15.2	28.4	13.2	14.6	60.8	46.2	147	160
18	61	1	2	16.3	19.3	3.0	10.2	31.6	21.4	144	39
Mean				19.0	28.0	8.7	11.2	37.4	26.2	304	94

TT = Thyrotoxicosis, STH = Thyrohypophysial syndrome.

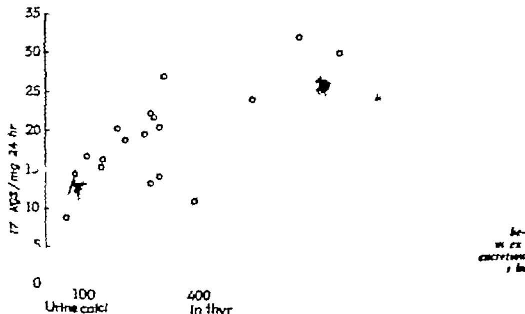
determined by clinical criteria and the results of laboratory findings. Although this subdivision was made partly on a subjective basis it was thought to be of some value, especially because the evaluation was always done by the same investigator. No correlation could be noted, however, between the degree of hyperthyroidism or the STH on the one hand and the response to metopirone on the other hand.

The urinary excretion of calcium was on an average 304 mg/24 hrs and in 14 cases a value in excess of 200 mg/24 hrs, was observed. This confirms the fact that hyperthyroid patients often, although not invariably, have hypercalcuria. The serum calcium values, on the other hand, did not exceed normal limits in any case. A close correlation was noted between the urinary excretion of calcium and the excretion of 17-ketogenic steroids (Fig. 1).

There was also a rough correlation between the *degree of hyperthyroidism*, on the one hand and the urinary excretion of calcium, on the other hand (Fig. 2). No correlation could be noted, however, between the degree of STH and the excretion of calcium.

The excretion of urinary 17 KGS decreased in the euthyroid state consistently from a mean value of 19.6 mg to 11.2 mg/24 hrs. In every case, except for case 6, the response to metopirone was definitely higher in the euthyroid stage and the mean excretion of urinary 17 KGS in the euthyroid group was 37.2 mg/24 hrs as compared with 8.9 mg/24 hrs in the hyperthyroid group. The degree of hyperthyroidism in the untreated patients did not seem to be correlated to the response to metopirone, nor was there any correlation between the increase in the excretion of 17 KGS following the administration of metopirone and the persistence of eye symptoms.

The excretion of urinary calcium decreased *following treatment* in all except



three patients (cases 9 10 and 14) and the decrease seemed to be directly correlated with the severity of the hyperthyroid state. In the patients classed as having severe hyperthyroidism the calcium excretion decreased from a mean of 375 mg to a mean of 90 mg/24 hrs, whereas in mild hyperthyroidism the calcium excretion fell from a mean value of 191 mg to 90 mg/24 hrs.

Four cases of hypothyroidism receiving adequate substitution therapy with thyroid hormones showed a normal response to metopirone and a normal excretion of calcium. No cases of untreated hypothyroidism were included in these series.

Discussion

The close correlation noted between the urinary excretion of calcium and 17 ketogenic steroids in hyperthyroid patients is perhaps the most important finding of the present investigation. As far as we know such a correlation has not previously been reported and the increased turnover of adrenocortical steroids which is known to occur in hyperthyroidism must therefore be taken into consideration as one of the factors on which the increased excretion of calcium in these patients depends. The fact that hypercalcuria cannot be demonstrated in all cases of hyperthyroidism remains unexplained however and it seems likely that additional factors are involved in the regulation of calcium metabolism in hyperthyroid patients.

The increased excretion of 17 KGS which was observed in the hyperthyroid patients during the course of the present study is in accordance with our previous findings (14) It should be noted, however that although the mean excretion of urinary corticosteroids in the present as well as in the previously reported series of hyperthyroid patients was found to be

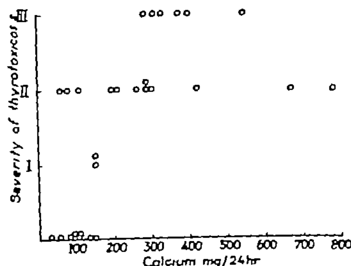


Fig. 2. Correlation between urinary calcium excretion and the degree of hyperthyroidism.

definitely increased, there was a marked difference in the excretion of 17 KGS in individual patients, which seemed to be unrelated to the degree of hyperthyroidism. It has been suggested that, in addition to the observed quantitative changes in the excretion of urinary corticoids, qualitative differences in the excretion pattern of adrenocortical steroids may also occur in these patients, urinary metabolites being produced which cannot be detected by the methods usually employed for the determination of urinary corticoids. It could therefore be postulated that the absence of hypercalciuria in some hyperthyroid patients can possibly be attributed to similar derangements of the metabolism of adrenocortical hormones.

The low response to administration of metopirone noted in most of the hyperthyroid patients included in the present study is consistent with the observations of Gold *et al.* (13) in three hyperthyroid patients and those of Brownie and Sprunt (4) in 13 cases of hyperthyroidism in whom the response to metopirone was in general poor. Some of the patients studied by the last mentioned authors showed virtually no response to metopirone but a good response to ACTH, while others responded fairly well to both metopirone and ACTH and one patient responded poorly to both. In order to determine whether the poor response to metopirone could be due to inadequate dosage they chromatographed the urine of the hyperthyroid patients before and on the second day following metopirone administration. On the second day the thyrotoxics excreted much less tetrahydro-11-deoxycortisol (THS) than do normal subjects, while at least some of the patients exhibited a normal fall in tetrahydrocortisol and tetrahydrocortisone, which would suggest a diminished reserve of pituitary corticotropin.

It should further be noted that in their original study on the clinical application of the metopirone test Liddle *et al.* (22) pointed out that in any one subject the increase of the oral metopirone dose from 500 mg to 750 mg every four hours resulted in only a slight increase of response and the dose-response difference was small compared with the differences in response which distinguished normal subjects from those with a so-called limited pituitary reserve. Similarly in a study on the effect of metopirone on the cortisol secretion rate, Lazarus *et al.* (20) observed that administration of metopirone more frequently than 500 or 750 mg every six hours did not result in any improvement of the response. It would thus seem that although it is possible that the dosage of metopirone used in the present study did not effect a complete inhibition of 11 beta hydroxylation in the adrenal cortex in every case, the differences in the response noted in the different groups included in the study are nevertheless significant and that the low response noted in many patients with untreated hyperthyroidism reflects a diminished reserve of pituitary corticotropin in these patients. This would mean that the increase utilization and turnover of adrenal hormones in hyperthyroid patients are the factors mainly responsible for the observed increase in the excretion of adrenocortical steroids in some of these patients and that a simultaneous increase of pituitary ACTH activity

if present at all, is not demonstrable under these circumstances. Excessive secretion of thyroid hormones would tend, as pointed out by Gold *et al.* (15) to deplete pituitary corticotropin, since the more rapid turnover of cortisol might require continued secretion of corticotropin in an effort to maintain normal circulating levels of cortisol, thereby leaving the pituitary with little or no corticotropin in reserve. On the other hand, one might still expect to find signs of increased pituitary corticotropin activity in those cases of exophthalmos which are not in a hyperthyroid state. A careful investigation of such cases with the help of more refined techniques, such as direct estimations of circulating ACTH levels, would obviously be of great interest.

Four cases of adequately treated hypothyroidism studied during the course of the present investigation showed a normal response to metopirone and a normal excretion of urinary calcium. Although we did not have an opportunity to study untreated cases of hypothyroidism, it should be noted that Liddle *et al.* (23) and Browne and Sprunt (4) have observed a poor response to metopirone in hypothyroid patients, which probably can be explained by a failure of the adrenal cortex to respond to endogenous corticotropin. Felber *et al.* (1) have similarly observed a poor response to exogenous corticotropin in certain cases of hypothyroidism.

Summary

The average excretion of 17 ketogenic steroids (17 KGS in a group of 70 hyperthyroid patients was found to be increased to 19.3 mg/24 hrs.

The excretion of urinary calcium was frequently although not invariably increased in the hyperthyroid patients, the average excretion of this group being 304 mg/24 hrs. There was a close correlation between the excretion of urinary calcium and urinary 17 KGS in the hyperthyroid patients and a rough correlation between the hypercalciuria and the degree of hyperthyroidism. Following treatment, the hypercalciuria disappeared and the decrease seemed to be directly related to the severity of the previous hyperthyroid state.

No correlation between the excretion of calcium and the persistance of exophthalmos (STH) following treatment of hyperthyroidism could be noted.

The response of the 17 KGS to the oral administration of metopirone (500 mg every 4 hrs) in the same group of patients was low and an average increase in the excretion of 17 KGS consisting of 8.9 mg/24 hrs was noted. On subsequent examination in euthyroid stage the mean basal excretion of 17 KGS in the same patients had decreased to 11.2 mg/24 hrs and the mean response to metopirone was at this time 37.2 mg/24 hrs. No definite correlation could be found between the response to metopirone on the one hand and the severity of the hyperthyroid stat or the occurrence of exophthalmos on the other It was concluded that the excessive utilization and turnover of adrenal hormones in the hyperthyroid stage probably results in a depletion of th pituitary corticotropin reserve which returns to normal when an euthyroid stage has been reached following adequate treatment.

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Cushing's Syndrome Co-existing with Hyperthyroidism

Report of a Case and Some Metabolic Observations

By

B A. Lamberg

In reviews on the pathology of Cushing's syndrome thyroid function is stated to be normal (13 73 80 81) The simultaneous occurrence of hyperthyroidism and Cushing's syndrome seems to be extremely rare. There are two cases on record, one with exophthalmic goitre and one with hyperthyroidism and nodular goitre (7 11) and in addition one case of Cushing's syndrome has been reported with concomitant malignant exophthalmos with euthyroidism (64) Two more cases, so far unpublished have come to my knowledge (59) Cushing's syndrome has also been reported to have developed after thyroidectomy in two instances (6 37) Owing to the rarity of the combination it has been deemed of interest to report on a case recently seen at the First Department of Medicine University of Helsinki, and on the metabolic tudies carried out in this case.

Case Record

The patient, peasant wife, aged 48, had no relevant family history She is the second of nine children; one sister has goitre. The menarche occurred at 12 menstruations being regular and normal. She has two daughters aged 13 and 16. She had measles and rubella in childhood. After the first delivery in 1946 she developed nephropathy with oedemata and the second delivery in 1949 was hindered by large myomata and Caesarean section was performed. A the same time sterilization was carried out. Two years before admission goitre was observed.

The family has farm in Central Finland. In the autumn of 1961 the patient' husband was hospitalized for several weeks for gastric ulcer and during that time the patient had to undertake greater amount of heavy work than usual. I October she started to have most disturbing insomnia and in spite of pronounced fatigue she would wake up at 2 a.m. and only fall to sleep again after some hours. She grew very tired and felt dizzy and as if she were intoxicated. She often felt pains in the middle of the chest which did not increase on effort. A the end of November 1961 she observed increasing growth of hair on the

alpha$_2$ 10.4 %, beta 12.5 and gamma globulin 18.1 %. Plasma creatinine 1.32 mg/100 ml. Thyroid antibodies: complement fixation and haemagglutinin: 0. The electrocardiogram was normal. Some data are shown in Table I.

Radiological examinations. The lungs were normal, the left ventricle of the heart appeared slightly enlarged. Intravenous pyelography gave normal findings. Retroperitoneal insufflation: the right adrenal gland appeared slightly larger than the left, no tumour was evident. Skull and sella turcica: normal.

The results of the steroid analysis are shown in Table II.

As a part of another project, the degradation of ^{131}I-labelled albumin was studied. A dose of 30 μc of ^{131}I-labelled albumin (Abbott Lab., Inc. Chicago, Ill. U. S. A.) was given intravenously and the distribution of radioactivity in the serum and urine was followed for ten days. The thyroid uptake of radioactive iodine was blocked by giving 10 drops of potassium iodide (5 % solution) 3 times daily. A control subject was studied parallelly. The results were analysed according to the method of Nosslin and Vetter (84) (Table III). During the first 7 days on potassium iodide the pulse rate decreased markedly from 90 to 54/min and returned to the pretreatment level again 10 days after the treatment was stopped.

With the intention of treating the Cushing's syndrome first with total adrenalectomy and later on the hyperthyroidism with radioactive iodine the patient was given 30 mg of carbimazole daily divided into 4 doses at 6-hour intervals, and 10 drops of potassium iodide (5 % solution) 3 times daily. She was discharged from the hospital under this treatment.

Second admission. June 4 to July 6, 1962. In the interval the patient's condition had improved to some extent. The tremor had disappeared, there was no transpiration, and she stated that she felt stronger. The weight had decreased, however, to 62 kg. On admission her pulse rate was 80–90/min, and regular. After two days on the ward she abruptly established fibrillation, with a heart rate of about 100/min and behaved somewhat oddly which indicated that she had still not turned euthyroid. She also felt some unexplained fear. She was given diazepam, meprobamate and chlorpromazine in addition to the previous treatment and the situation subsided within a few days. The fasting blood sugar remained between 70–90 mg/100 ml and there was at this time a constant slight glucosuria of 0.5–1.0 per cent glucose in the urine. Serum cholesterol had increased to 299 mg/100 ml, sodium 144 meq./l, potassium 3.5 meq./l. Blood pressure 190/100 mm Hg.

Another study of the steroids was made when she was still not fully euthyroid. Metopyrapone was given intravenously 15 mg of the bitartrate/kg body weight as an 8-hour infusion (45). Urine was collected in 8-hour portions, starting at midnight; the infusion was administered between 8 and 16 hrs (Table IV). The dexamethasone suppression test (35) and an intravenous cortisol disappearance test with 1 mg of cortisol/kg of body weight as a 30 min i.v. infusion (41, 43) were performed (Table IV).

She remained about 4 weeks in the ward and gradually became euthyroid. She was transferred to the II Surgical Department (Head: Prof. V. Seiro). Operation was carried out in two seances (Dr. M. Turunen). The right adrenal gland was removed on July 17. It weighed 7 grams. The left adrenal gland was removed on July 28, its weight was also 7 grams. The histological appearance was normal and the width of the adrenal cortex was about 2 mm.

Third admission. August 3 to August 31, 1962. After the operation she was maintained on hydrocortisone 50 mg, desoxycorticosterone 10 mg and carbimazole 20 mg daily. Pulse rate 80/min, regular. Blood pressure 170/100. Fasting blood sugar 68 mg/100 ml, sodium 136–144 meq./l, potassium 3.1–6.0 meq./l, calcium 9.2 mg/100 ml, phosphate 2.9 mg/100 ml. There was still inconstant glucosuria. The ocular fundi were normal. The thyroxine-binding capacity of the serum (thyroxine-binding index) (50) was 100 μg/100 ml, i.e. slightly elevated.

In order to evaluate the dose of radioactive iodine to be given as a treatment, a radioiodine test was performed 39 hours after removing the carbimazole block. The uptake (24 h) was 45 %, the excretion 52 % on the first day and 3 % on the second day. She was given 10 mc of radioactive iodine 50 hours after discontinuance of the block. The carbimazole treatment was resumed 48 hours after the administration of the radioiodine for further 6 weeks. The patient was discharged from

the hospital, being maintained on 50 mg cortisone acetate and 10 mg DCA per day. Before discharge, the blood pressure was 150/90 in recumbency and 133/90 in the upright position.

Fourth admission. November 6 to November 17 1962. In the interval the patient had been doing better. The beard had disappeared and the hair begun to grow again on the forehead. Her strength had increased considerably. Her weight was 64 kg and blood sugar 34—100 ml; there was no glucosuria. The oral glucose test was normal and no glucose appeared in the urine. Pulse rate 80/min, regular blood pressure 150/90. PBI 8.7 μg/100 ml, cholesterol 284 mg/100 ml, radioactive iodine test: uptake (24 h) 51 %, 48 hours excretion 27 %, conversion ratio (24 h) 49 %, PB^{131}I (72 h) 0.51 /l. Electrocardiogram normal. X-ray of the chest revealed that the heart was slightly smaller than on the previous occasion.

Fifth admission. April 18 to May 21 1963. Her condition had in the meantime improved considerably. She was now able to carry out the work on the farm to almost the same extent as before the disease had started. On effort she had some pains in the shoulders and in the chest but not of anginal type. The beard had totally disappeared, the hair on the scalp was growing better (Fig. 2) and the former striae were now pale, although the epidermis was still very thin and there was still some pigmentation. Weight 69.5, BMR + 2 %, PBI 6.0 μg/100 ml, radioiodine test: uptake (24 h) 42 excretion 32 % on the first day and 6 % on the second day conversion ratio (24 h) 32 %, PB^{131}I 0.362 %/l. Serum proteins 8.1 g/100 ml, albumin 58 alpha$_1$ 4.0 %, alpha$_2$ 7.5 %, beta 10.5 % and gamma globulin 20.0 %. The thyroid now appeared to be about 25—30 g in size, rather firm and somewhat uneven

Table II. Data on corticosteroids during second admission.

Successive days	Urinary excretion 17-keto-steroids mg/d	Urinary excretion 17-hydroxy steroids mg/d	Plasma corticoids, μg/100 ml 9.00	13.00	15.00	17.00
1	21.6	50.3	31.3			29.5
2	15.1	36.5	42.1			
3 (ACTH)	54.6	78.6	53.4	79.5	93.3	
		Metopyrapone test, 15 mg/kg as 8-hour infusion				
1st day						
0.00-8.00	6.0	11.9				
8.00-16.00	9.0	48.8	21.3			33.1
16.00-24.00	10.3	72.3				
Total	25.3	133.3				
2nd day	16.9	72.9				
		Dexamethasone suppression test				
1	11.6	34.7	Dexamethasone 0.5 mg × 4			
2	8.4	31.8	— — — —			
3	4.9	12.1	— — 2.0 mg × 4			
4	3.8	3.1	— — —„—			

Hydrocortisone: 1 mg/kg T½ = 83 min.

on the surface, with a firmer area low in the right lobe. Thyroid radioiodine scanning revealed that the radioactivity accumulated in the right lobe and the isthmic area more abundantly than in the left lobe. The fasting blood sugar was 60 mg/100 ml, and there was no glucosuria. Intravenous glucose tolerance: k = 2.15. Thyroxine-binding index 110 µg/100 ml.

On admission her serum calcium was 11.5 mg/100 ml and rose within few days to 12 1 The phosphate was 4.5 and 3.5 mg/100 ml. The daily calcium excretion was 100—190 mg/day Tubular reabsorption of phosphate was 88.9 % On intravenous calcium tolerance test (35) there was a normal decrease in phosphaturia (from 700 to 350 mg/day) and the calcium retention was 73.5 %. The patient evidently developed *hypercalcaemia of the postadrenalectomy state or Addison disease* (31 65, 86) and the substitution therapy was immediately changed to 9-alpha-fluorohydrocortisone 0.2 mg/day and cortisone acetat 50 mg/day Within 7 days the hypercalcaemia disappeared: serum calcium was 10.5 and phosphate 3.8 mg/100 ml.

On the third admission, when the patient was still not fully euthyroid, and on the fifth admission

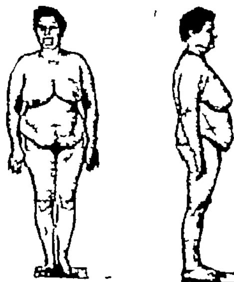


Fig 2 May 1963

blood samples were drawn and lyophilized. The lyophilized material was sent to Dr J M. McKenzie who carried out TSH assay according to his method (57 58) The first sample was tested twice, in the autumn 1962 and parallelly with the second sample. In both samples the long acting thyroid stimulator (LATS) was present (1 57 58) and on the first assay of the first sample possibly also TSH of the normal or short acting type (2)

Discussion

The relationship between the adrenal and thyroid glands has long been the object of intensive studies and for literature the reader is referred to several reviews (14 28, 40 53 63 72) It is by now well known that clinical hyperthyroidism accelerates the disappearance of glucocorticoids from the blood (8, 12, 43 54 69 70 71) In animal experiments the administration of thyroid hormones increases the secretion of cortisol but the response is lacking after hypophysectomy At the same time the disappearance of corticosteroids also increases (62) The conjugation of the steroids is accelerated (8) and the metabolism is characterized by an increased oxidation of the 11 hydroxy groups to 11 ketones in contrast to the situation in Cushing's syndrome and after stimulation with ACTH (23 31) In addition, the 5-alpha-reductase is stimulated (23 31) An increased level of ACTH like material in the blood of hyperthyroid patients has recently been observed, which may be brought about by the aberration of the steroid metabolism (33) It is also of interest to note the depressed response to ACTH (19 42, 62 a) and metopyrapone (9 23 a) and the decreased responsivity of the adrenal pituitary axis to otherwise suppressive doses of dexamethasone (22) suggesting

the existence of overactivity in this system. During treatment of hyperthyroidism the eosinophil count usually increases (52) and hyperthyroidism also affects the circadian rhythm of the plasma cortisol level (60).

On the other hand it is known that the glucocorticoids influence thyroid function in an inhibitory way although the exact mechanism is still obscure. This also happens in hyperthyroidism (87). Hyperthyroidism in association with Addison's disease has been reported in some 20 cases and the incidence has been estimated to be higher than in a normal population (28). For pertinent literature the reader is referred to some recent reviews (10, 17, 21, 26, 28, 83, 87). According to the hypothesis proposed by Harris and Woods (28), the lack of adrenal response to stressful stimuli may induce hyperthyroidism through inadequate adjustments in the adrenal-thyroid-pituitary system. It is of interest to note that ACTH increases the thyroid uptake of radioactive iodine after adrenalectomy in man but not in the normal subject (67).

In summary, it appears that thyroid hormones in excess increase adrenal function, although the patients do not usually exhibit any cushingoid features presumably because of the deviation of steroid metabolism with formation of less active steroid compounds (31, 33). (In the author's experience, however, the profound cushingoid rounding of the cheeks, not seldom encountered in Graves' disease with the exophthalmic syndrome, especially in patients of post pubertal age and in young women, appears striking.) On the other hand, excess of glucocorticoids tends to suppress thyroid function. *In clinical hyperthyroidism* prednisolone decreases the rate of radioiodine accumulation in the thyroid and decreases the level of PBI (67). *In Cushing's syndrome* excess of tri-iodothyronine has been shown to increase the excretion of 17-hydrocorticoids to levels not usually seen in hyperthyroidism (23). Furthermore, glucocorticoids probably exert extrathyroidal effects, since cortisone counteracts the increase in oxygen consumption in hypophysectomized rats treated with thyroxine and inhibits the increase of ^{32}P release from the erythrocytes of the animals (5). In thyroid crisis, the effect of glucocorticoids, which may be life-saving when given in conjunction with other therapy considered specific in this condition, probably includes more than mere reduction in thyroid function and supplementation of adrenal hormones (48, 85).

Hence, the co-existence of hyperthyroidism and Cushing's syndrome may induce rather complicated metabolic adjustments. In the literature there are, to the best of my knowledge, only two cases so far reported. In 1955 Cazzola (11) described a female patient, aged 26, who suffered from Cushing's syndrome. She was treated with pituitary irradiation and Graves' disease subsequently developed. In 1956, Bickel *et al.* (7) described another case, a female of 36, who within 4 years developed acromegaly, Cushing's syndrome and hyperthyroidism. In this case there was no clear-cut evidence of the exophthalmic syndrome and the thyroid was nodular. A third case should be added to this list. In 1958, Morgan and Mason (64) reported on a female patient, aged 39, who very rapidly development malig

nant exophthalmos with euthyroidism and a few months later Cushing's syndrome. This must be regarded as a combination of Graves disease and Cushing's syndrome but without hyperthyroidism. The patient committed suicide and at autopsy the thyroid appeared otherwise normal but contained an adenoma with microfollicular structure. McKenzie (59) has observed two more cases which have not yet been published. Soffer *et al.* (81) found diffuse and nodular goitre in several patients, increased BMR in some, and elevated PBI in one, but tests of thyroid function usually gave normal results and there was no clinical evidence of hyper or hypothyroidism. Bevan and Thornton (6) described a case of mixed adrenogenital syndrome and Cushing's syndrome in which hyperthyroidism developed shortly after thyroidectomy and Hurxthal and Musulin (37) have published a similar case. At least three cases of Cushing's syndrome associated with thyroid carcinoma have been described (15 38, 74) The 3 male patients with hyperthyroidism, gynaecomastia and deranged glucose metabolism without clinical Cushing's syndrome reported by Rosenthal and Lees (75) should probably not be included in this group, but may represent some border line condition.

In surveys of large series of Cushing's syndrome the thyroid function has been reported to be normal (13 73 80 81) It is of interest, however to note the occurrence of exophthalmos-producing activity in the blood and pituitary of patients with exophthalmos and Cushing's syndrome (78) since some degree of exophthalmos is rather frequently encountered in Cushing's syndrome (73 81)

Some observations in the present case may be shortly discussed

Glucose metabolism is known to be affected by changes in thyroid function, although the results are rather contradictory (18) The fasting blood sugar level is frequently slightly elevated (3 18) and there is sometimes glucosuria (3) The oral glucose tolerance often shows a high peak with rapid removal (14) although diabetes-like curves have also been reported (3) The renal threshold for glucose has been thought to be decreased (3) The elevation of the fasting blood sugar may be due to a variety of reasons including, for instance (18) increased breakdown of glycogen, increase in insulin degradation, increased absorption from the intestine, *etc* The finding of increased levels of ribonuclease in the blood of hyperthyroid patients has been interpreted as possibly indicating an increased release of insulin (52) The hyperglycaemic response to intravenous infusion of glucagon is significantly less in hyperthyroidism than in normal individuals (49) indicating an increased break down of the glycogen stores in conformity with animal experiments. Owing to the fact that a variety of techniques have been used for the determination of glucose utilization the results of studies on hyperthyroidism are rather conflicting (18) Elrick *et al.* (18) were not able to demonstrate any difference in utilization rate between hyperthyroid and euthyroid subjects. The k value in the present case, 1 15 was at the lower limit accepted as normal in this clinic and coincided with the lowest value seen in a group of hyperthyroid patients with a mean k value of 1.80 (49) On the other hand glucocorticoids are

known to decrease the k value. Later on, at the fifth admission, the k value was 2.45 indicating that in the active phase, at least, glucose utilization was less than when the patient had recovered. This would seem to indicate that in the active stage of the combined diseases the excess of glucocorticoids was dominant.

The disappearance of intravenously administered cortisol was markedly more rapid than in normal subjects (41) The $T\frac{1}{2}$ of 83 min. is in conformity with previous observations made with the same method (45) It seems that in this respect the thyroid condition was dominant, since the rate of disappearance of cortisol in situations with altered adrenal function, including Cushing's syndrome, has usually been shown to be normal (12, 25 30, 71) This is also in conformity with the observations of Gabrilove and Weiner (21) in a case of concomitant Addison's disease and hyperthyroidism in which the disappearance rate varied with the thyroid function.

The study with ^{131}I-albumin indicated a daily degradation of about 22 g This was 70 higher than the control case studied at the same time (and other control cases) In spite of this considerable loss the serum protein level was normal indicating an adequate increase in protein synthesis. It seems that in this respect the excess of thyroid hormones dominated as compared with the effect of adrenal steroids. The negative nitrogen balance in hyperthyroidism is well known and studies with radioalbumin have shown an increased turnover of albumin (76, 79) The effect of glucocorticoids is known to increase the breakdown of injected albumin (27 77) whereas in Cushing's syndrome the matter is probably more complicated (34 82) possibly owing to the action of simultaneously increased androgenic compounds. However the exchangeable albumin pool is decreased (34 82) as is also seen in the present case, but this may also be the result of excess of thyroid hormones (76)

The response to intravenous metopyrapone was unusual, in that the urinary excretion of 17 hydroxy-corticosteroids increased to 193 mg during the test day whereas intravenous ACTH brought about an increase to only about 80 mg In hyperthyroidism the response to metopyrapone has been shown to be less than normal (9 23 a) and even in Cushing's syndrome the response to metopyrapone should probably not profoundly exceed that to exogenous ACTH (20) Although this is only a single observation, an explanation seems to be required. The deviation of the steroid metabolism from the normal pattern, shown to exist in hyperthyroidism (23, 31) in conjunction with the hyperresponsivity known to exist in Cushing's syndrome without adenoma may have some bearing on this matter Moreover the pronounced increase in 17-hydroxy-corticosteroid excretion that occurred in one case of Cushing's syndrome after administration of triiodothyronine (23) does not correspond, either to the usual patterns of steroid excretion seen in clinical hyperthyroidism. Grant (24) has pointed out that metopyrapone may also have a direct stimulatory effect on 11 deoxycortisol production in the adrenal cortex. Hence the appearance of a dissociation between the response to ACTH stimulation and to metopyrapone block may be related to an increased sensitivity

produced by Cushing's syndrome in conjunction with an increased production of adreno-cortical hormones common to both conditions and the alteration in the metabolic pathways of steroid metabolism occurring in hyperthyroidism. This problem could possibly be further elucidated by studies on patients with Cushing's syndrome during administration of thyroid hormones.

On both occasions suppression with dexamethasone elicited a response regarded as typical of adrenal hyperplasia. Fractionation of 17 ketosteroids revealed nothing spectacular

The thyroxine-binding index was slightly elevated, the normal range being 45–80 μg/100 ml (50) However the elevation at the PBI was not solely due to the increase in TBIx, since the location in a correlation diagram (50) deviates from what would be expected with such a level of TBIx. The reason for this elevation remains obscure, however and it may be mentioned that an increase in TBIx has previously been found in a number of female patients with thyroid disease (50)

The question then arises whether the hyperthyroidism in this case was a symptom of Graves disease or whether it was merely a manifestation of toxic nodular goitre. Initially the thyroid appeared non-nodular although there was some unevenness on the surface which may have been due to moderate lobulation. The thyroid scanning carried out after radioiodine treatment is, of course, difficult to evaluate, but no distinct local accumulation could be observed, although the distribution was somewhat uneven which might have been brought about by the treatment. There were no eye signs suggestive of even a slight exophthalmic syndrome (47) The TSH assay revealed the presence of LATS in the blood assumedly not present in toxic adenoma but again these samples were drawn 3 months after thyrostatic treatment and 8 months after radioiodine therapy respectively which may have induced changes in LATS activity The author is, however inclined to regard the hyperthyroidism as being that of Graves disease.

If one accepts the view that strong emotional stimuli may induce both Graves' disease and Cushing's syndrome, a matter that is still debatable (36) then one could visualize a common hypothalamic-pituitary background The hyperthyroidism evidently developed shortly after Cushing's syndrome became manifest, as judged from the PBI and cholesterol values determined a few months apart. The clinical appearance of the hyperthyroidism may have been delayed, however through the action of excess adrenal hormones on thyroid function (87) The clinical manifestations of the hyperthyroidism were also to some extent masked and the original suspicions were confirmed by the pronounced depression of the pulse rate during the administration of potassium iodide in connexion with the ^{131}I albumin study There was some pigmentation unusual in Cushing's syndrome (16) in which condition it may indicate the presence of a pituitary adenoma (61) or carcinoma (29) In hyperthyroidism such pigmentation, in the author's experience, is sometimes really intense, and it has been shown that the disappearance of intravenously admi

nistered cortisol is even more rapid in hyperthyroid patients with pigmentation (46). In the present case it was probably a manifestation of increased ACTH and MSH production, pointing to a central origin of the condition. The author is inclined to accept the view of Cazzola (11) and of Bickel *et al.* (7) that a common unknown hypothalmic pituitary functional lesion may have been the basic lesion. Since the simultaneous occurrence of Graves and Cushing's syndromes seems to be extremely rare in spite of the rather common incidence of the former and since thyroid function has been shown to be normal in Cushing's syndrome, the combination of the two conditions, if not purely coincidental, would probably involve some unknown basic alterations in the regulation of the production and metabolism of hormones present only in a small number of individuals.

Summary

The case of a female patient aged 48, with concomitant Cushing's syndrome and hyperthyroidism is presented. The clinical picture was dominated by the hyperactivity of the adrenal glands. The most notable signs were hirsutism, striae and moderate brown pigmentation, which was thought to be due to increased production of ACTH and MSH. The patient lost weight and did not appear very obese. The clinical signs of the hyperthyroidism were masked by those of Cushing's syndrome. But as regards metabolism, studies with intravenously administered cortisol and ^{131}I albumin revealed that the thyroid condition was the dominating feature. The disappearance rate of intravenously administered glucose was within the lower range of normal variation. It was thought that a common hypothalamic-pituitary derangement may have been the basic lesion in this case. The patient was treated with total adrenalectomy after having been brought to euthyroidism with thyrostatic drugs and potassium iodide, and later on the hyperthyroidism was combatted with radioactive iodine. A TSH assay performed after adrenalectomy on two occasions revealed the presence of the long acting thyroid stimulator.

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Thyroxine Binding Globulin in Thyroid Disease Determined on Acetylated Cellulose

By

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In the early 1950's it was shown that thyroxine was firmly bound to a protein moving between α_1 and α_2-globulins on electrophoresis in addition to being more loosely bound to the serum albumin. Barbiturate buffers were usually used and occasionally some binding to the pre-albumin area was observed. The problems related to the binding of thyroid hormones to serum and other proteins have been recently extensively reviewed (9, 11, 21, 22, 25, 27).

Under physiological conditions practically all thyroxine in the serum is bound to the thyroxine binding inter-alpha globulin (TBG), although small amounts also seem to be bound to the pre-albumin (TBPA) (8, 11). It seems that endogenous variations in the protein-bound iodine in the serum (PBI) are normally mainly related to changes in the binding capacity of TBG. The binding capacity of TBPA is higher than that of TBG but little is known of the physiological role of this protein (8, 9, 12, 17). Recently Ingbar (10) has found however that TBPA at physiological pH is able to carry a significant amount of thyroxine and hence may play an important rol in the peripheral metabolism of the thyroid hormones. The binding capacity of TBG and TBPA changes to some extent with the level of thyroid function (11). Thus the binding of thyroxine to TBG seems to decrease in hyperthyroidism and to increase in hypothyroidism (23).

In a large population of patients with thyroid disorders there always seems to be some overlapping of the PBI values of patients from groups representing different levels of thyroid function (15, 16). The elucidation of the causes of such discrepancies requires further laboratory testing, e.g. determination of the non-butanol extractable iodine (NBEI) by chemical or radiochemical means, radio-chromatography of the serum iodine compounds, determination of the binding

the pre-albumin occurred, because barbiturate inhibits the binding to TBPA (6 7 10, 28)

The point of equal binding to albumin and TBG was obtained in the following manner: the ratio of thyroxine bound to albumin to that bound to TBG was calculated for each of the four (three) samples and plotted on linear graph paper against the total thyroxine concentration. The best straight line was drawn through the points. The "thyroxine-binding index (TBIx) was obtained from the graph and represents the concentration of added thyroxine at which the ratio is 1.0 (Fig 1) plus the endogenous thyroxine concentration calculated from the PBI value.

Results

Since a close linear correlation seemed to exist between the PBI values and the TBIx in the *control* group a regression analysis of all these data was made (Fig 2) The distribution of the values for the control subjects was in fact found to fit a straight line corresponding to the formula $y = 17.8 + 5.6x$ ($r = 0.82$ standard error of the mean $y_{x_0} = 4.9$ μg/100 ml. y = TBIx in μg/100 ml, x = thyroxine concentration in μg/100 ml)

The sera drawn from patients with *non-toxic goitre* showed a more variable pattern (Fig. 3) In two cases the TBIx and PBI were slightly decreased. In several cases the TBIx exceeded the upper normal limit and similar changes were observed in the PBI values. Since in an endemic goitre region like that in

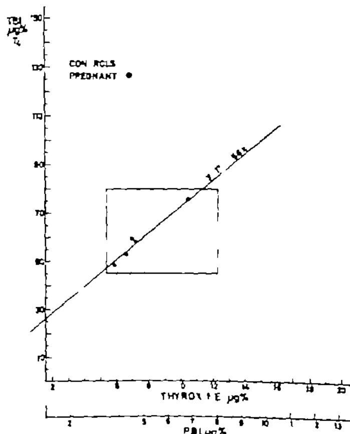


Fig The correlation between the TBI (expressed as μg 100 ml thyroxine) and the PBI or the corresponding endogenous thyroxine concentration in the control subjects and three pregnant women. Open circles, females; filled circles, males. Block indicates the normal variation in TBI and PBI

Finland (14) the uptake of radioactive iodine by the thyroid is usually elevated the finding of a PBI above the upper normal limit in a goitre patient requires further elucidation and the determination of TBIx may be of importance. This is illustrated by the findings in one such case (Table I) a female patient, 52 with a slightly enlarged thyroid gland originally suspected of having hyperthyroidism because of elevated PBI values.

The TBIx values in the group of non toxic goitre patients in whom increased amounts of NBE ¹³¹I were observed were also normal in all cases and the PBI elevated in one case only

Several of the patients rendered euthyroid by treating their hyperthyroidism with radioactive iodine still had elevated PBI values as previously observed (5) The TBIx values also were increased in several of them, as is seen in Fig 3 The elevation of the PBI values was in some cases correlated to an increase in TBIx.

Table I. Data on the iodine metabolism in an euthyroid goitre patient with elevated TBIx not due to pregnancy

^{131}I uptake by the thyroid, 24 hrs		75 %
^{131}I excretion in the urine, 24 hrs		25 %
PB^{127}I		11.2 µg %
PB^{131}I 72 hrs		0.15 %/l
Non butanol extractable ^{131}I		10 %
Radiochromatography of serum, 72 hrs		T_4 100 %
TBIx		145 µg %
T_3-test (8 days, 100 µg/day)		
PB^{127}I	before	10.0 µg %
	after	4.2 µg %
Urinary excretion of ^{131}I		
	before: 1st day	21.0 %
	2nd day	1.0 %
	after 1st day	51.0 %
	2nd day	8.0 %
Restudied one year later		
PB^{127}I		8.8 µg %
TBIx		130 µg %

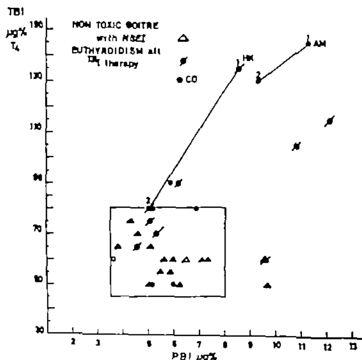


Fig 3 The correlation between the TBIx and PBI in non-toxic goitre patients and in a few subjects previously hyperthyroid but successfully treated with radioactive iodine (crossed symbols) Numbers indicate the order of determination (cases H.K. and A.M.) in both patients with one year interval. Case C O had subacute thyroiditis and infectious mononucleosis. Open symbols, females, solid symbol males. Black indicates the normal variation of TBIx and PBI

In one case the elevation seemed to be due to the appearance of NBEI after treatment (male patient with a PBI of 9.6 µg/100 ml and normal TBIx in Fig 3) since with the radioactive technique about 30 % of the PB[131]I was not extractable with butanol.

In the *hypothyroid* group (Fig 4) a correlation between TBIx and PBI values similar to that found in the control group seems to exist, but the line seems to have moved towards lower PBI values. In at least three of these cases the normal PBI could be attributed to an elevation of TBIx. In three cases with normal PBI the TBIx was also normal. The shift to the right from a fictitious regression line could suggest the possible occurrence of NBEI but this could not be studied. In one of these cases the hypothyroidism was due to radioiodine treatment for hyperthyroidism which is known sometimes to induce an elevation of NBEI.

The absence of any correlation between PBI and TBIx in the *hyperthyroid group* is striking when compared to the general trend in all other groups of patients (Fig 5). This group also included one case of *thyreotoxicosis factitia* due to excessive ingestion of desiccated thyroid. The values appear to fall into two groups: one with low normal values for TBIx and a few values below the lower normal limit and another in which the TBIx values are all above the upper normal limit. In the first group the PBI values were normal but only in four of them could the low PBI possibly have been due to a decrease in TBIx. In the second group with high TBIx values there were two cases with normal PBI values, the reason for this discrepancy remains obscure.

In all graphs, male patients have been indicated by solid symbols, whereas in the female cases these have been left open. When looking at the figures the high TBIx values found exclusively in *female patients* with thyroid disorders is striking. The number of male patients is, of course, much smaller but none of them had increased levels of TBIx.

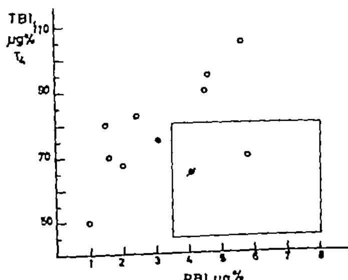


Fig 4 The correlation between the TBI and PBI in hypothyroid patients. The cases falling outside the block of normal variation include also two nontoxic goitre patients with the iodine binding (peroxidase) defect and one case of pituitary dwarfism with adrenocortical, gonadal and thyroid insufficiency. Open circles females, solid circles males.

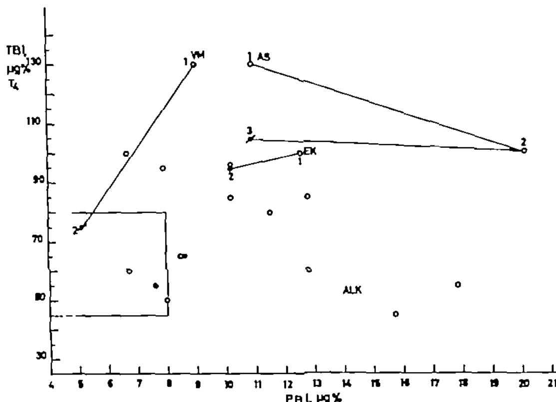


Fig 5 The correlation between the TBIx and PBI in hyperthyroidism. The numbers indicate order of determination. in case E.K the interval was 2 months in case V.M 1 year and in case A.S 7 months and 2 years, respectively Case A.L.K. had thyrotoxicosis factitia. Crossed symbols euthyroidism after treatment with radioactive iodine open circles females and solid circles males. Black indicates the normal variation in TBIx and PBI

Discussion

It has been found difficult to estimate the absolute binding capacity of TBG by means of conventional electrophoresis with barbiturate buffer (19 20) When the thyroxine level was increased, the binding of hormone to TBG also seemed to increase continuously This phenomenon was thought to be due to the adsorption of albumin-bound thyroxine to the paper (21) The use of reverse flow electrophoresis with barbiturate buffer (18, 19) or conventional electrophoresis with another buffer (tris-maleate, bicarbonate) (9) seems to yield better results.

Using cellulose acetate strips we found no evidence of trailing in experiments with thyroxine and albumin. The saturation level of the serum TBG could be established in most cases, but in a number notably those in which TBIx appeared elevated, the amount of thyroxine bound to TBG seemed to increase continuously with increasing thyroxine concentration in a manner similar to that reported by Robbins and Rall (21) (Fig 1) The reason for this is not clear It may however be observed that TBG appears not to be a homogenous compound (1 6 7 26 28)

For this reason the expression of the thyroxine binding capacity of TBG in terms of a saturation level was abandoned, and instead another parameter of the

binding the TBIx, *"thyroxine binding index"* was used for the same purpose. As judged from the close correlation between PBI and TBIx values in the control group, the use of this method seems to be justified and from a practical point of view it aids in the elucidation of discrepancies found in PBI determinations.

The considerable number of *female* patients with *thyroid disease* who showed elevated TBIx values, is striking. In several of the female patients treated for hyperthyroidism with radioactive iodine the TBIx was elevated and the PBI accordingly high. In two cases with subacute thyroiditis, one tested during the acute phase and the other one year after the acute phase, the TBIx was also above the expected range. Although no apparent correlation seems to exist between this elevation of the TBIx and thyroid disease in female patients this situation seems to warrant further study. The increase of TBIx in a number of female patients between 60 and 70 years of age would seem to exclude variation in the oestrogen level as the determinant factor. A similar trend was seen in a number of normal female subjects by Tanaka and Starr (23, 24).

From the observations in cases of nontoxic goitre it is apparent that endemic goitre is not associated with any characteristic variations in TBIx. When the PBI values are corrected for the presence of NBEI in that special group where increased proportions of NBE ^{131}I were observed, resulting PBI values will be fairly low which indicates that these people really live very close to the hypothyroid level, although they are clinically euthyroid.

Summary

The binding of radioactive thyroxine to thyroxine binding globulin (TBG) was measured with the "saturation" method by using electrophoresis, veronal buffer and acetylated cellulose. The binding capacity was expressed as an "index" (TBIx) indicating the total thyroxine concentration at which the binding to albumin and to TBG was equal. In normal persons there was a linear correlation between the PBI and TBIx values. The same was observed in a group of hypothyroid subjects, in some of whom a normal PBI could be explained on the basis of an elevation of TBIx. In hyperthyroidism the general trend seemed to be towards lower levels, although only in a few cases could a normal PBI be attributed to a low TBIx. In a number of female patients with thyroid disease there was an elevation of TBIx, the cause of which remained unknown.

Acknowledgements

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The Effect of a Two-day Treatment with Chlorothiazide on the Urinary Excretion of Calcium, Phosphate and Sodium in Hyper and Hypocalcaemia

By

P. Torsti and B. A. Lamberg

The effect of chlorothiazide on the urinary excretion of calcium has been little studied. The available evidence, although scanty, seems to suggest that the thiazides initially tend to enhance calciuria. Van Dommelen (9) reported an initial increase in one and no changes in two other patients on chlorothiazide. In short term experiments, Hänze (19) and Lichtwitz *et al.* (27) observed a transient increase in calciuria. Similar observations were made by Glaubitt and Rausch-Stroomann (17) in the initial phase of a more prolonged treatment.

Lamberg and Kuhlbäck (23) observed originally that *after* a two-day administration of chlorothiazide and hydrochlorothiazide calcium excretion declined to very low levels. The decrease began already on the second day of treatment but was marked on the following day and persisted for a number of days after the treatment. The available data did not point to any involvement of the parathyroid glands, there were no changes in the tubular reabsorption of phosphate and no sufficient changes in the glomerular filtration rate to account for the diminished calciuria. The conclusion was reached that the tubular reabsorption of calcium was increased.

These observations were later confirmed by others. Lichtwitz *et al.* (27) conducted a large-scale study with a variety of thiazides and observed a constant decrease in calciuria after treatment in both hypercalcaemic and hypercalciuric patients. The same was seen in more prolonged studies by Glaubitt and Rausch-Stroomann (17) these workers also found that the decrease started during the treatment.

Chlorothiazide has also been shown to produce an increase in the urinary excretion of phosphorus (17) although inconstantly (23). In short term experiments Ford *et al.* (15) observed no significant changes.

Since the mechanism by which chlorothiazide influences calcium excretion especially *after the treatment, remained* obscure, further studies have been carried out during the last few years. The project was subdivided into three parts, the first (a) concerning the effect of chlorothiazide in hypo- and hypercalcaemic states; since a certain parallelism seemed to exist between the behaviour of sodium and calcium, studies were also carried out (b) in diseases of the adrenal gland, and finally in order to elucidate the role of purely renal changes (c) in a group with renal disease. The present report deals with part (a) and a subsequent paper with part (b) (24)

Material and Methods

The patients comprised 7 cases with hyper calcaemia and/or hypercalciuria and 6 patients with hypocalcaemia. Previously reported cases (23) and few additional subjects without renal, adrenal or parathyroid disease served as controls.

The 7 cases of hypercalcaemia and/or hyper calciuria included 4 patients with primary hyper parathyroidism due to parathyroid adenoma (cases 1 to 4) one of whom had concomitant hyperthyroidism (35), one patient with hyper calcaemia due to multiple myeloma (case 5), one in whom hyperparathyroidism was suspected owing to prolonged elevation of the serum and urinary calcium but with negative findings on surgical exploration, [later on the calcium values gradually decreased (case 6)], and one patient with unexplained hypercalciuria (case 7)

The cases of hypocalcaemia comprised two patients with postoperative hypoparathyroidism (cases 8 and 9) one with slightly decreased serum calcium studied immediately after removal of parathyroid adenoma (case 10) two patients with the malabsorption syndrome (cases 11 and 12) one of whom showed only hypocalciuria, and one patient with hypoproteinaemia and probably malabsorption (case 13 In case 12 there was evidently osteomalacia, since the alkaline phosphatases were elevated and there was 90 per cent calcium retention on intravenous calcium tolerance test, and, furthermore, probably secondary hyperparathyroidism, since there was no change in the phosphaturia during the same test.

In most cases the patients were kept on a low calcium diet containing no milk, cheese or fish. During the last year of the study however a calcium-phosphorus diet was ordered, providing 300 mg of calcium and 800 mg of phosphate daily

The standard procedure was as follows: Chlorothiazide 1.0 g twice daily was administered orally for two days. Blood was drawn every day and urine collected daily for some days before the test, during the test period and a few days thereafter Calcium, phosphorus, sodium, potassium and creatinine were measured from the blood and the urine. The complete programme could not be followed in all patients, however

Calcium was estimated by EDTA titration according to Vikkila *et al.* (30) inorganic phosphorus according to Fiske and Subbarow (14) sodium and potassium were assayed with flame photometer and creatinine by the method of Benedict and Behre (1).

Results

The results are compiled in Tables I and II and in Figs. 1—4 The response may be divided into two phases: 1) changes seen during treatment and 2) in the period following termination of the treatment.

Urinary calcium. In the first phase a small increase in the calciuria could be observed in a few instances in the control patients and in the hypercalcaemic subjects but the changes were inconstant. In the cases of hypocalcaemia, however *there was in most cases an increase in the* calciuria (Fig 1) From the data available it seems that an increase is usually to be

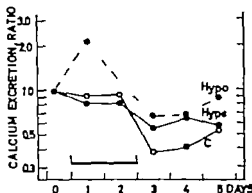


Fig. 1 The urinary calcium excretion during and after chlorothiazide treatment as compared to the pre-treatment level (1.0) in hypocalcaemic (Hypo), hypercalcaemic (Hyper) and in 5 control subjects (C). Treatment period indicated by horizontal bar

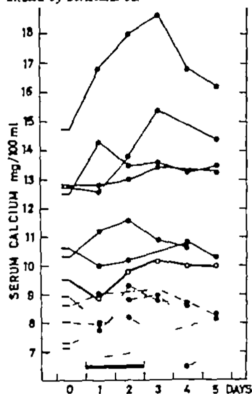


Fig. 3 The serum calcium during and after chlorothiazide treatment in patients with increased serum or urinary calcium (•———), with decreased serum or urinary calcium (•- - - -) and in 3 control subjects (o——o). Treatment period indicated by horizontal bar

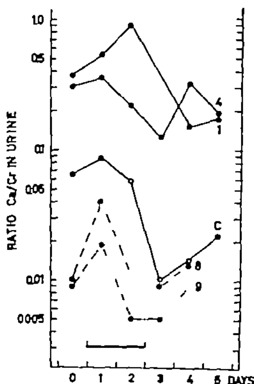


Fig. 2. The urinary calcium/creatinine ratio during and after chlorothiazide treatment in hypercalcaemic (cases 1 and 4), hypocalcaemic (cases 8 and 9) and one control subject (C) Treatment period indicated by horizontal bar

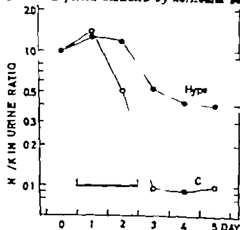


Fig. 4 The urinary Na/K ratio during and after chlorothiazide treatment as compared to the pre-treatment level (1.0) in hypercalcaemic patient and one control subject (C). Treatment period indicated by horizontal bar

Table I. Excretion of calcium, phosphorus, sodium, potassium and

Case No.	Substance[1]	Days 1	2	3	4	5[2]	6	7	8	Remarks
1	Ca	1100	779	571	696	546	416	189	299	Hyperpara-
	P	1400	1450	1520	1120	1900	1220	1860	1370	thyroidism
	Na	135	148	198	113	356	193	95	29	
	K	72	51	47	71	105	107	143	78	
	Cr			1500	1500	600		1260	1620	
	V	2900	3400	3300	2900	3900	2600	2900	2250	
2	Ca	222	337	329	176	275	272	371	288	Hyperpara-
	P	380	495	529	551	847	419	403	432	thyroidism
	V	1900	2250	2300	2800	2200	1550	1550	1800	
3	Ca		568	680	358	468	320	320	380	Hyperpara-
	V		2350	2700	2650	2250	1600	1600	1900	thyroidism
4	Ca		581	534	633	445	249	320	324	Hyperpara
	P		1062	388	750	1480	1045	918	962	thyroidism
	Na		33	70	310	154	7	4	16	
	K		54	46	113	125	50	41	38	
	Cr		1950	1700	1800	2010	1940	1000	1690	
	V		1200	1600	2500	1600	1100	900	1300	
5	Ca		360	134	132	120	49	181	37	Myelomatous
	P		510	450	650	609	625	625	504	hypercalcaemia
	Na		157	46	192	78	20	12	4	
	K		55	47	67	81	41	40	28	
	Cr		810	810	910	720	550	380	567	
	V		1500	1500	2600	1460	1250	1250	1050	
6	Ca		294	224	304	245	158	126	198	Suspected hyper
	P		573	608	633	716	708	640	573	parathyroidism
	Na		51	18	122	80	33	29	18	
	K		48	44	87	72	49	49	46	
	V		1250	650	2250	800	600	650	600	
7	Ca			432		500	297	334	222	Hypercalciuria
	P			108	123	1237	633	750	770	of obscure
	Na			104	30	224	63	91	83	origin
	K			69	80	95	65	68	61	
	Cr			1440	1560	2400	1240	1440	1159	
	V			1180	2250	1350	650	750	900	
8	Ca	11	150*	12	34	12	9		12	Hypopara-
	P	644	735	620	650	430	297		351	thyroidism
	Na			42	228	91	65		44	
	Cr			1040	800	900	1000		870	
	V	1400	2450	400	2000	1200	900	600	650	

creatinine in urine after oral administration of chlorothiazide

Case No.	Substance	Days 1	2	3	4	5	6	7	8	Remarks
9	Ca			13	30	8	8	15		Hypopara
	P			528	930	930	902	694		thyroidism
	Na			34	228	46	14	14		
	Cr			1470	1580	1650	1680	1690		
	V			800	1750	1100	700	750		
10	Ca		59		41	28	27	35	42	After removal of
	P		798	766	950	1080	384	1080	1170	parathyroid
	Na			177	247	247	118	218	202	adenoma
	K			73	110	104	98	64	40	
	V		2000	2200	2650	2700	1600	2000	2100	
11	Ca			25	9	8	2			Malabsorption
	P			226	436	440	639			
	Na			93	203	75	12			
	Cr			610	730	1000	900			
	V			350	1300	800	450			
12	Ca	3	55[*]	7	14	12	7	2	7	Malabsorption
	P	441	534	350	780	667	702	744	690	
	Na			98	332	170	31	21		
	V	700	1100	1000	2000	1700	900	800	700	
13	Ca	40	35	23	130	63	22	18	16	Hypocalcaemia,
	P	136	672		1111	1281	1100	1080	880	malabsorption?
	Na	115	126	35	516	363	70	37	27	
	V	1050	600	600	2900	3050	1100	600	800	

Daily excretion of calcium, phosphorus and creatinine (Cr) are expressed as mg per day sodium and potassium as meq per day urine as ml per day (V)

Days 4 and 5 = treatment period.

Intravenous calcium tolerance test.

expected when the urinary calcium excretion is below 100 mg a day The small increase was more evident when th calcium excretion was correlated to the creatinine excretion (Fig 2)

In the second phase a marked decrease was observable in all the subjects tested except the one w th the lowest basal excretion. It is of course to be expected that such a decrease will not be evident unless the base line is sufficiently high to allow a decrease to occur E en in the cases with a relatively low basal excre tion however th trend was fairly evident. This response persisted for 2–3 days.

Serum calcium. There was a trend towards an increase in the serum calcium level but this was not a constant phenomenon. (Table II Fig 3) It was evident, however in cases with primarily elevated serum calcium and especially in case 1 with the highest baseline level. This elevation

Table II. Serum calcium, phosphorus, sodium, potassium

Case No.	Substance[1]	Days 1	2	3	4	5[2]	6	7	8
1	Ca	13.2	15.6	15.4	16.8	18.0	18.7	16.8	16.2
	P	1.5	3.3	2.3	2.6	4.0	3.8		3.3
	Na		137	135	144	141	137	136	141
	K		4.8	4.7	4.5	3.5	3.6	3.1	2.7
	Cr				1.6	1.8	1.7	1.8	1.7
2	Ca		12.7	12.3	14.3	13.5	13.6	13.3	13.5
	P		2.2	1.8	2.3	2.5	1.8	2.7	2.7
4	Ca		12.8		12.8	13.0	13.5		13.3
	P		2.8		3.3	3.2	3.0		2.7
	Na		124		128	128	136	135	141
	Cr		0.9		1.1	1.2	1.3		
5	Ca			12.7	12.6	13.8	13.4		14.4
	P			4.0	4.3	4.7	4.[illegible]		3.6
	Na			138	141	137	137		124
	K			3.8	3.3	4.1	3.8		3.2
	Cr			1.1	0.9	1.1	1.0		0.9
6	Ca		10.3	10.3	11.2	11.6	10.9	10.7	
	P		2.4	3.0	2.5	2.8	3.0	2.9	
	Na		139	139	139	141	141	144	
	K		4.1	3.9	4.4	4.5	3.6	2.6	
7	Ca		10.7	10.4	10.0	10.2		10.8	10.3
	P		3.5	5.0	3.3	5.0		4.5	4.2
	Na		139	143	148	141		143	141
	K		4.5	4.4	4.7	3.7		3.8	4.1
	Cr		*0.9*	*0.9*	*1.0*	*1.0*		*1.2*	1.1

persisted, in those cases in which it was evident, for some days after treatment was discontinued. With a baseline above 10 mg/100 ml a rise seems to be expected.

Urinary sodium and potassium. In most cases the usual increase in sodium excretion was seen in period I accompanied by an increase in potassium excretion in those cases in which potassium was determined. After cessation of treatment there was a profound decrease in sodium excretion, the potassium excretion fell to about the base-line level and the Na/K ratio exhibited a marked depression which persisted for several days (Fig. 4). In cases 1 and 4 the effect on potassium excretion was more pronounced. Both had primary hyperparathyroidism. In both cases the excretion rose to exceptionally high levels and in case 1 the increase persisted for longer than usual.

Serum sodium and potassium. No marked

and creatinine after oral administration of chlorothiazide

Case No.	Substance[1]	Days 1	2	3	4	5[2]	6	7	8
8	Ca		6.1	8.5	7.8	8.8	9.0	8.1	
	P		5.3	4.6	3.5	3.5	4.1	3.3	
	Na			133	132	138	142	140	
	Cr			1.2	0.9	0.8	1.0	0.8	
9	Ca			8.6	9.0		9.2	8.7	8.5
	P			2.9	3.6		4.2	3.4	3.0
	Na			138	137		131	143	140
	Cr			0.9	1.1		1.3	1.1	1.1
10	Ca			8.9	8.0	9.3	8.8	8.7	
	P			2.7	2.5	3.0	3.0	1.7	
	Na			146	146	137	148	141	
	K			6.3	3.2	4.6	3.5	5.0	
11	Ca				9.5	10.5	10.0	9.7	
	P				3.9	4.0	4.9	4.1	
	Na				143	142	130	139	
	Cr				0.8	0.7	1.0	0.	
12	Ca		7.2	6.9	6.8		7.1	6.5	6.9
	P		3.9	4.1	3.3		5.3	4.8	4.9
13	Ca	8.0	8.0		8.0	8.2	7.6		8.2
	P	5.0	3.0	3.4	2.6	2.8	3.3		4.0
	Na	142	134	122	136	122	137		133

Serum calcium, phosphorus and creatinine are expressed as mg/100 ml, sodium and potassium as meq/litre.

Days 4 and 5 = treatment days.

changes were observed in the serum sodium level during and after the test period. Unfortunately serum potassium was measured in only a few cases. In case 1 in which there was a prolonged kaluretic response there was a marked decrease in the serum potassium level, the lowest value being reached on th third day after the test. A similar response was observed in case 6 and a slight decrease in case 7

Urinary and serum creatinine showed no clear-cut changes during or after the test.

Urinary phosphate In many cases a trend towards an increase in the serum phosphorus level was seen but these changes were rather inconstant. Owing to possible variations n phosphate intake the evaluation should be restricted to those cases in which the patients were maintained on a constant phosphorus diet (Cases 4 9 12 13) In all these

cases (one of hyperparathyroidism three hypocalcaemia) the increase in phosphat uria was marked It started during the treatment and continued for a few days thereafter

Serum phosphate The serum phosphate remained unchanged in most instances but in the cases of hypocalcaemia there was a decrease during the first day of the test period concomitantly with the in crease in phosphaturia.

Discussion

When calcium excretion is considered, the course of events has to be divided into two different periods 1) the treatment period (I) and 2) the period following discontinuance of treatment (II)

The increase in calcium excretion in period I was on the whole rather small and inconstant when measured in absolute terms as mg/day This may be the reason why the increase was proportion ally most evident in those cases in which the base-line excretion of calcium was low With higher levels of excretion a small increase may not be visible. The increase was apparent in most cases in which the daily excretion had initially been below 100 mg of calcium. By what mechanism such a reaction can occur can only be conjectured The main questions would seem to be a) is it a direct renal action of the drug b) is it in some way related to extrarenal drug action? or c) is it only a passive phenomenon induced by other urinary changes? The exact mode and site (or sites) of action of the thiazides are still not fully elucidated These problems have been discussed in a number of recent reviews (2 25 31 45) According to current views, the thiazides mainly inhibit sodium reabsorption in the proximal tubule. The increased tubular content of sodium in the lower portions of the nephron provides more sodium to be exchanged for potassium and enhanced potassium excretion will ensue. The exchange mechanism is dependent on mineralocorticoids (43) and inhibited by spironolactones (34) and hence the action of thiazides on the exchange is dependent on mineralocorticoids (11 16, 34) There is also some evidence that thiazides affect other sites in the nephron (10 15 20 31 33 38) With regard to renal handling of calcium, it seems that calcium reaches the tubule partly by glomerular filtration partly by extraglomerular influx (3 32) It is reabsorbed to 97—99 per cent (7 22 28) in the tubule and there is evidence for reabsorptive sites in the proximal tubule (7 37) as well as in the distal portions, according to studies with the stopped-flow technique (18 32 43) For further details the reader is referred to recent reviews (12 21 36)

If the increase in calcium excretion in period I were due to direct renal action on calcium reabsorption similar to that postulated for sodium, a more marked effect would have been expected. Thus it would seem somewhat far-fetched to regard the increase as due to a direct renal action of the drug In some cases with initially high serum calcium level there was a further increase which persisted even after the treatment had been discontinued. This might possibly indicate an extrarenal action on the bone although this explanation seems to be at variance with the findings of Lichtwitz

et al (27) who observed a positive calcium balance and no changes in the serum calcium. The matter seems to warrant further investigations. At all events such a reaction would induce an increased filtered load of calcium but available methods did not allow a more detailed study of the filtration and tubular reabsorption of ionized calcium. Finally osmotic diuresis induces increased calcium excretion (7 40, 44) whereas water diuresis does not (46) The simplest explanation would then be that the osmotic diuresis induced by the thiazide is responsible for the slight increase in calcium excretion. This explanation is also in accordance with the findings of Hänze (19) and of van Dommelen (9)

After these initial changes the situation as regards calcium excretion gradually passes into another phase. In our previous study (23) as well as in the present report a decrease in calcium excretion was often already evident during the second day of treatment. The same has been reported by Lichtwitz *et al.* (27) and Glaubitt *et al.* (17) It seems that as soon as the initial changes are induced, an adjusting and adaptive mechanism starts to function. Hence we suppose that the changes seen during a somewhat longer treatment represents two effects, one induced directly by the drug and the other consisting of an adaptive reaction. The latter would then be most pronounced immediately after cessation of the treatment (period II)

The second period is characterized by a marked decrease in calcium excretion. This occurs in all categories of patients irrespective of the initial level of excretion, except in those who had a very low base-line excretion. This is, of course, understandable, since to be evident the decrease in calcium excretion requires at least a minimal level from which to fall. This reaction persisted for two or more days. In the light of our previous observations and according to Lichtwitz *et al.* (27) the decrease is brought about by increased tubular reabsorption of calcium. There is a remarkable similarity between the course of the sodium excretion and the urinary Na/K ratio (Figs. 1 2 4) The profound decrease in sodium excretion after cessation of treatment was also pointed out by Castle *et al.* (6) who regarded this reaction as indicating the activation of a sodium conservation mechanism. It has recently been pointed out that there is a certain correlation between the renal handling of calcium and sodium (41) It has even been suggested that sodium and calcium may have a common binding site in the tubule for which they compete (41) and that maybe other earth metals share this property and compete with sodium transport (36) The matter will be discussed further in a subsequent paper (24)

It may also be of interest to note that in one case of hyperparathyroidism there was an unusually prolonged kaliuretic response during which the serum potassium decreased markedly In another case of suspected hyperparathyroidism, although the diagnosis could not be proved, there was a marked hypokaliaemic response.

Hypokaliaemia seems to be an unusual feature of hyperparathyroidism (5 29) A pronounced response as regards serum potassium is seen in hyperaldosteronism (8) and it has been shown that the kaliuretic response is proportional to the

amount of mineralocorticosteroids available for activation of the sodium-potassium exchange in the distal tubule (11, 16). It thus seems possible that in some cases of hyperparathyroidism a state of relative potassium depletion may occur which possibly might be due to secondary aldosteronism. This, on the other hand, could be induced by sodium depletion, since it has been shown that injection of calcium and of parathyroid hormone also increase sodium excretion (4, 26, 59).

The changes in phosphate excretion were less marked but in those cases in which a constant phosphate diet was taken an increasing trend could be observed. This started on the second day of treatment and continued during the ensuing days and may therefore reflect the inverse relationship which is known to occur between calcium and phosphate.

Summary

The effect of a two-day treatment with chlorothiazide on the urinary excretion *of calcium, sodium and phosphate and on* the urinary Na/K ratio was studied in patients with elevated or depressed urinary or serum calcium. The response after cessation of treatment was similar to that previously observed in normocalcaemic subjects. During the treatment an increase in calcium excretion was observed in hypocalciuric patients and an increase in the serum calcium level in hypercalcaemic patients. The phosphate excretion increased slightly. After cessation of the treatment there was a profound decrease in the excretion of calcium and sodium and in the Na/K ratio. A certain degree of parallelism seemed *to exist as regards* the behaviour of calcium and sodium. This to some extent confirms the similarity in the renal handling of those ions reported in the literature.

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second study was performed during maintenance treatment with 50 mg hydrocortisone daily and 1.5 mg d-aldosterone (divided into 3 equal doses and administered at equal intervals intramuscularly) daily.

Case 4. Totally adrenalectomized for Cushing disease. Female, aged 48. (Originally this patient had also had hyperthyroidism, which was treated with radioactive iodine (24). At the time of study she was euthyroid). During the chlorothiazide study she was maintained on 0.2 mg 9-fluorohydrocortisone and 30 mg hydrocortisone daily. In this patient a spironolactone test was also carried out during the same treatment.

Case 5. Female, aged 29. She supposedly had developing hypopituitarism. The daily urinary excretion of 17-ketosteroids, 17-hydroxy-corticosteroids, oestrogens and gonadotrophins was 3.7 mg, 3.7 mg, 50 mg and below 10 mouse units, respectively. During stimulation with ACTH the excretion of 17-ketosteroids and 17-hydroxy-corticosteroids rose to 8.3 and 27.5 mg, respectively. The response to intravenous methopyrapone (Metopirone[R] Ciba) (15 mg per kg of body weight given as an 8-hour intravenous infusion) was practically absent; the excretion of 17-ketosteroids before the test was 1.3 and during infusion 3.5 mg, whereas the respective values for the 17-hydroxicorticosteroids were 3.2 and 4.7 mg.

She was considered to have only slight, if any, impairment of mineralocorticoid reactivity.

Case 6. Male, aged 59. Limited adrenocortical reserve due to recurrent pulmonary infections. The excretion of 17-ketosteroids and 17-hydroxy-corticosteroids was 2.2 and 3.5 mg per day, respectively, and after ACTH stimulation the respective values were 3.5 and 12.5 mg per day. An oral methopyrapone test (750 mg six times

Table I. Excretion of calcium, phosphorus, sodium, potassium

Case No.	Substance[1]	Days 1	2	3	4[2]	5[2]	6	7	8	Remarks
1	Ca		46	48	62		50	54	39	Addison
	P		735	744			1000	1195	698	disease
	Na		88		158	252	239	157	115	cortisol
	K		69		74	99	86	84	56	
	V		2100	2400	3000	2550	2650			
2	Ca		75	120	118	187	198	175	172	Addison
	P		504	576	456	756	720	882	675	disease
	Na		112	189	118	358	162	156	216	cortisol
	K		68	67	57	77	82	78	75	
	V		1200	1200	1200	1800	1750	1750	1500	
3	Ca	47	33	30	33	36	41	51	40	Addison's
	P	550	520	770	1533	624	770	777	494	disease
	Na	135	92	110	116	160	118	155	134	cortisol
	K	37	33	62	70	55	74	58	64	
	Cr	1010	980	990	1090	940	910	1110	910	
	V	1100	1000	1100	1400	1050	1375	1850	1300	
3a	Ca	48	57	54	40	90	51	55	67	cortisol +
	P	486	200	300	378	490	652	552	492	d-aldosterone
	Na	58	83	38	284	293	83	61	58	
	K	49	38	32	61	59	46	35	41	
	Cr	1500	1080	1090	940	1230	1500	910	1060	
	V	935	1250	600	1800	1750	720	950	1200	

daily during one day) brought about an increase of similar magnitude.

Case 7. Male, aged 56. The patient had been treated continuously for 3 years with prednisolone for bronchial asthma. H was considered to hav minimal responsivity if any as regards glucocorticoid production whereas the reactivity with regard to mineralocorticoids was considered to be fairly normal. The chlorothiazide test was carried out during prednisolone treatment (30 mg/day).

The chlorothiazide treatment was carried out as previously described (25, 43) 1.0 g being given twice daily for two days. I addition, spironolactone (Aldactone[R] Searle) was administered for two days, 200 mg 4 times daily to six subjects: case 1 control subject, case 2, hyperparathyroidism, cases 3 and 4 hypoparathyroidism, case 5, hypocalcaemia of other origin. Case 6 in this series is identical with case 4 in the chlorothiazide series (totally adrenalectomized patient)

Blood and urine were analysed before, during and after the respective tests as previously described (43).

Results

Studies with chlorothiazide (Tables I and II, Figs. 1—3)

Urinary calcium. During the treatment period (I) there were no significant changes except in two patients: in case 2 an increase occurred which persisted during period II and in case 7 a small increase was observed as in patients without adrenal impairment (25, 43)

and creatinine in urine after oral administration of chlorothiazide.

Case No.	Substance	Days 1	2	3	4	5	6	7	8	Remarks
4	Ca		86	83	92	71	36	35		After bilateral adrenalectomy cortisol + 9-α-fluoro-cortisol
	P		784	536	624	756	864	772		
	Na		97	77	222	282	77	83		
	K		68	54	88	63	34	42		
	Cr		1590	1150	1400	1400	1160			
	V		950	800	1600	1800	800	800		
5	Ca		199		199	63	119	152	163	Hypopituitarism
	P		635	652	984	698	608	492	345	
	Na		82	112	211	120	43	38	54	
	K		52	42	91	74	38	22	27	
	Cr		910	890	1340	900	1000	890	900	
	V		1700	1650	2400	1500	1330	1200	1500	
6	Ca	95	85	93		78	56	98		Limited adrenal reserve
	P	500	455	377	384	347	413	583		
	Na	37	59	68	269	143	64	64		
	K		32	57	75	53	57	70		
	Cr	930	819	913	1090	620	990	1130		
	V	500	450	550	1600	700	700	1650		
7	Ca		176	154	206	122	67	75		After prolonged prednisolone treatment
	P		625	602		1048	568	933		
	Na		121	73	112	107	27	7		
	Cr		1580	1280	1600	1500	1020	1430		
	V		850	680	1500	800	400	490		

Urine calcium, phosphate and creatinine (Cr) are expressed as mg per day sodium and potassium as meq per day urine volume as ml per day (V)

Days 4 and 5 = treatment period.

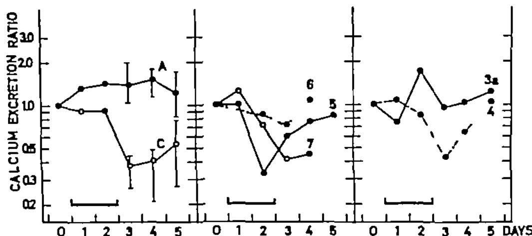


Fig 1 The urinary calcium excretion during and after chlorothiazide treatment as compared to the pre-treatment level (1.0) in patients with Addison's disease (A cases 1 to 3) in 5 control subjects (C) and in cases 3a to 7 (see table I). The range of variation in groups A and C is indicated by vertical bars The treatment period is indicated by horizontal bar

The response after cessation of treatment (period II) is of considerable interest (Fig 1) In the *Addisonian patients* substituted with cortisol alone the mean excretion remained above the base-line level. In one of them (case 2) there was a considerable increase which already started during period I When in case 3 *d*-aldosterone was added to the substitution therapy no significant change in the response could be observed (3 a, Table I Fig 1) In contrast to this, in case 4 a patient who had been totally adrenalectomized and who was maintained on cortisol and 9-α-fluorocortisol, a decrease occurred after cessation of treatment (Fig 1) but the reaction was insignificant when the Ca/Cr ratio was considered (43) The same can be said of cases 5 and 6, whereas in case 7 the response could be described as normal.

The serum calcium did not change significantly although an increasing trend was clearly observable (Fig. 2)

Urinary sodium and potassium. In the *Addisonian patients* (cases 1 to 3) the excretion of sodium and the Na/K ratio increased during period I and remained elevated throughout period II (Fig 3) In this respect there seemed to be a certain parallelism between the behaviour of the Na/K ratio and the excretion of calcium. In case 3 when also substituted with *d*-aldosterone and in case 4 the reaction was modified in that an increase occurred during the treatment but the Na/K ratio fell almost to the base-line level in period II (Fig 3) In cases 5 and 6 the reaction in period II was very feeble; in case 7 however a profound decrease in sodium excretion occurred.

There were no clear-cut changes in the serum *sodium, potassium* and *creatinine* The *creatinine excretion* usually remained unchanged, only in two instances did a slight increase occur during treatment. In all patients there was an increase in *phosphate excretion* starting during treatment and persisting for some days thereafter

whereas there were no changes in the *serum phosphorus* level.

Urine volume increased very feebly in the Addisonian patients maintained on hydrocortisone and this increase usually occurred after treatment had ceased. In the other patients the diuretic response occurred during treatment as usually

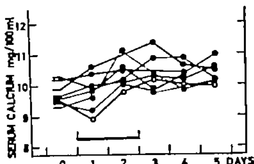


Fig. 2. The serum calcium during and after chlorothiazide treatment in patients with impaired adrenal function and in 3 control subjects (open symbols). Treatment period indicated by horizontal bar

The spironolactone series (Table II)

Urinary calcium. The calcium excretion increased slightly in all except case 5 in which the initial excretion level was lowest. In cases 1 and 2 the increase was marked and persisted for several days. The *serum calcium* did not change. The *excretion of sodium* increased to a variable extent and the changes in *potassium excretion* were very small. There were no noteworthy changes in *serum sodium*, *potassium* or *phosphorus*, whereas *phosphorus excretion* usually showed a slight decrease.

Discussion

The mechanism proposed in our previous paper (43) to explain the decrease in calcium excretion occurring after chlorothiazide treatment (period II) was

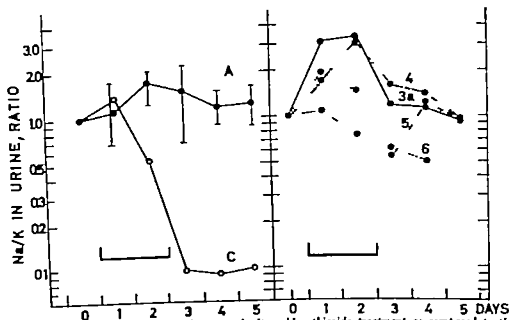


Fig. 3. The urinary Na/K ratio during and after chlorothiazide treatment as compared to the pre-treatment level (1.0) in patients with Addison disease (A) (cases 1 to 3), in cases 3a to 6 and in one control subject (C). Treatment period indicated by horizontal bar

Table II. Serum calcium, phosphorus, sodium, potassium and creatinine after oral administration of chlorothiazide

Case No.	Substance[1]	Days 1	2	3	4[2]	5[2]	6	7	8
2	Ca		10.7	9.9	9.9	10.2	10.9	10.9	10.2
	P		3.8	4.1	4.3	4.5	4.3	4.4	3.5
	Na		137	130	137		139	133	137
	K		5.9	5.5	5.4	5.3	5.5	4.5	4.8
3	Ca	9.7		9.7	10.0	10.1	10.3	10.2	10.6
	P	3.2		3.9	3.5	3.3	3.8	3.8	3.5
	Na	143		136	134	137	131	128	125
	K	4.6		4.7	4.7	4.7	4.8	4.3	4.7
	Cr	0.9		0.8	0.8	0.8	1.0	1.1	1.0
3a	Ca	9.7	10.3	10.6	10.4	10.5		10.4	11.0
	P	2.6	3.0	3.3	3.0	3.6		3.1	3.0
	Na	140	131	134	148	134		140	143
	K	4.3	4.8	4.8	4.5	3.6		3.6	4.6
	Cr	0.8	0.8	0.8	0.8	0.8		0.9	0.8
4	Ca		10.4	9.3	10.6		11.4	10.7	
	P		3.5	4.7	3.8		5.3	4.0	
	Na		138	138	136		131	145	
	K		5.3	4.7	4.7		4.4	4.3	
	Cr		1.2	1.2	0.9		1.0	0.8	
5	Ca	10.0	9.6	9.3	9.9	10.6	9.9		10.1
	P		3.6	3.7	4.0	4.0	4.3		3.9
	Na		148	140	137	140	147		131
	K		5.1	4.3	4.8	4.8	4.4		5.0
	Cr		0.8	0.8	0.8	1.4	0.9		0.7
6	Ca		9.1	9.5	9.6	11.1	10.4	9.9	
	P		3.2	3.9	3.7	4.3	3.9	4.1	
	Na		148	134	140	134	137	142	
	K		4.8	5.1	4.7	4.7	4.8	4.8	
	Cr		0.8	1.0	0.8	1.0	1.1	1.0	
7	Ca		9.2	9.8	9.2	10.1	9.8	10.4	
	P		3.6	3.9	3.6	4.3	3.5	2.7	
	Na		145	149	146	139	137	137	
	Cr		1.2	1.3	1.0	1.2	1.1	1.2	

[1] Serum calcium, phosphate and creatinine are expressed as mg/100 ml, sodium and potassium as meq/litre.

[2] Days 4 and 5 = treatment period.

Table III. Excretion of calcium, phosphorus, sodium, potassium and creatinine in urine after oral administration of spironolactone

Case No.	Substance[1]	Days 1	2	3[2]	4[2]	5	6	Remarks
1	Ca	72	88	40	126	81	90	Normal person
	P	510	616	272	893	643	210	
	Na	8	44	32	60	49	38	
	V	750	1100	800	1750	900	600	
2	Ca		324	335	460	396	495	Hyperpara-thyroidism
	P		962	816	763	580	1038	
	Na		16	40	102	64	26	
	K		38	16	20	28	54	
	Cr		1690	1680	3740	1540	2040	
	V		1300	1350	1780	1150	1200	
3	Ca		12	36	30	35	20	Hypopara-thyroidism
	P		351	221	153	138	174	
	Na		44	46	53	84	26	
	Cr		870	990	790	1020	910	
	V		660	850	850	930	600	
4	Ca	14	17	12	19	20	13	Hypopara thyroidism
	P	463	513	819	648	675	528	
	Na		86	164	125	75	34	
	Cr		1260	1160	1630	1330	1470	
	V	700	600	1300	1200	750	800	
5	Ca	5	5	6	7	3	1	Hypocalcaemia, malabsorption
	P	726	525	625	649	658	539	
	Na	170	87	134	139	44	18	
	Cr	2610	1400	1500	1620	1360	1400	
	V	1200	950	1000	1160	850	650	
6	Ca	90	75	119	98	122	104	After bilateral adrenalectomy
	P	714	652	617	506	462	644	
	Na	58	35	143	183	209	176	
	K	43	37	30	50	46	54	
	Cr	1530	1140	1280	1340	1150	1950	
	V	600	430	900	1100	1200	1300	

Urinary calcium, phosphate and creatinine (Cr) are expressed as mg per day sodium and potassium as meq per day (V).
Days 3 and 4 = treatment period.

based on the superficial similarity of the response in the sodium and calcium excretion and in the Na/K ratio. It was suggested that possibly the renin-angiotensin-aldosterone mechanism, known to be activated by sodium depletion (3, 11, 12, 26, 41, 42), may be involved. A similarity in the renal handling of calcium and sodium has been reported (18, 47, 48). Calcium infusions increase the renal excretion of sodium (29, 46) and so does the administration of parathyroid hormone to hypoparathyroid subjects (6). A constant ratio between sodium and calcium has been observed in the renal tubules (28, 48) and in the absence of hyper- or hypocalcaemia a constant ratio between calcium and sodium clearance seems to be maintained (36, 47). Distal tubular sites of calcium reabsorption located in the vicinity of the sodium reabsorption sites have been revealed by the stopped-flow technique (16, 37, 49). It has been proposed that sodium and calcium possibly compete for some common binding sites in the tubular membrane (47) and speculations have also been made as to whether this could be applicable to other earth metals as well (45).

According to our previous observations (43) and to part of the present data, a certain superficial parallellism seems to exist between sodium and calcium excretion after treatment. In the Addisonian patients substituted with cortisol alone the excretion curve after chlorothiazide treatment is similar in shape as regards calcium excretion and the Na/K ratio (Figs. 1 and 3). In cases 5 and 6 a feeble response was seen in both respects. In the cases substituted with both cortisol and a mineralocorticoid the superficial resemblance seems to be lost.

As regards the calcium excretion after cessation of the treatment (period II) the decrease normally seen is not primarily dependent on the presence of glucocorticoids, since there was no response in the Addisonian patients although they were maintained on cortisol treatment. Furthermore, it may be significant that in case 7, a patient who had been treated with prednisolone continuously for 3 years and presumably lacked an adrenal reactive capacity as far as glucocorticoids were concerned, the reaction was absolutely normal. A permissive role of glucocorticoids is possible, however, since it is known that they have a permissive action with regard to the renal handling of water (2, 22) and minerals (14, 33, 44).

The exact role of the mineralocorticoids is more difficult to assess. In case 3 when *d*-aldosterone was added to the cortisol treatment no significant changes occurred in the response. In case 4, on the other hand, a patient who was maintained on 9-α fluorocortisol and cortisol, a reaction did occur although very small and of rather short duration. One more difficulty arises from the fact that it is at present impossible to give an exact definition of a normal response. In case 5, supposedly a case of developing hypopituitarism with little, if any, impairment of mineralocorticoid production, the reaction could also be classified as a normal one, whereas in case 6, with a limited adrenal reserve, the reaction was very feeble. There are, however, a few indications which may be taken as indirect evidence that a role is played by the mineralocorticoids.

As regards the Na/K ratio there was an increase during period II in all three Addisonian patients substituted with cortisol. In case 3 when *d*-aldosterone was added to the therapy and in case 4 the Na/K ratio, which increased more during period I fell to the base-line level in period II but did not decrease below that level in contrast to all the other subjects studied This would indicate that the sodium-conserving mechanism set in action after cessation of treatment (7) or probably even during the treatment, is primarily dependent on a normal adrenal reactive capacity as far as the production of mineralocorticoids is concerned. It is known that prolonged treatment with chlorothiazide in rats produces an increase in the granulation of the juxtaglomerular apparatus (41) indicative of increased renin production (42) which in turn activates the zona glomerulosa to secrete more aldosterone. In man, treatment with thiazides induces an increase in aldosterone excretion within a few days (20) and alterations in the sodium load produce changes in the plasma renin content (5) On the other hand, direct activation of the adrenal gland without mediation of the renin mechanism is also possible (4)

With regard to the calcium excretion in period II it may be noted that in most cases at least a superficial parallelism seems to exist between the calcium excretion and the Na/K ratio (Figs 1 and 3) Since the reaction in period II is evidently dependent on adrenal function and independent of the presence of cortisol, as seen in the patients with Addison's disease, it would seem logical to assume that the adrenal factor involved would be the mineralocorticoids. In some respects the observations are compatible with the hypothesis put forward. One difficulty arises from the different responses seen in cases 3 and 4 One has, however to consider a possible difference in mineral action between a daily oral dose of 0.2 mg 9-α-fluorocortisol and an 8-hourly intramuscular administration of 500 mg *d*-aldosterone, since the biological half-life of aldosterone is rather short. Furthermore, if a competitive binding really exists in the tubules the response would be influenced by the difference in loads as regards the competing substances. Although it seems highly probable that the reaction in the calcium excretion is dependent on mineralocorticoids, it is impossible at present to assess whether they have only a permissive role in this respect. Other mechanisms may also be involved. Angiotensin for instance, has a direct action on the renal handling of sodium (27 30 35 38, 39) which will be modified by aldosterone, but so far its effect on calcium excretion has not been studied In our opinion the fact remains that the decrease in calcium excretion in period II is dependent on mineralsteroids, although the mechanism by which these steroids are involved is not clarified by the present data.

In order to obtain further information, some tests with spironolactone were carried out. Along with a marked increase in sodium excretion and the Na/K ratio there was a slight increase in calcium excretion. Although feeble the effect on calcium excretion seemed to differ qualitatively from that seen during chlorothiazide treatment, since it was more prolonged. This could be interpreted as due to

another mechanism, similar perhaps to that known to govern sodium excretion, i.e. a counteraction of the effect of the mineralocorticoids (1 10 13 19 21 32 40) The matter requires further study

Preliminary studies with aldosterone administered intravenously to subjects with normal adrenal function have shown that a decrease in calcium excretion parallel with a decrease in the Na/K ratio occurs in about 50 / of these cases. The very fact that this reaction does not occur constantly would indicate the presence of other modifying factors in addition to aldosterone On the other hand, such an action is in contrast to that exhibited by glucocorticoids, which are known to increase calcium excretion by means of decreased tubular reabsorption (8 9 23 34)

In a previous study (43) an increasing trend in the serum calcium level was observed during the treatment (period I) This trend was most marked in patients with initially high serum calcium levels. It was speculated whether this reaction could be due to some extrarenal action, i.e an action on the bone. This observation was at variance to those of Lichtwitz *et al* (31) who observed a positive calcium balance during treatment without changes in the serum calcium. In the present series a similar increasing trend was observable, although the changes were small (Fig 2) The reaction, whatever its mechanism seems not to be dependent on adrenal function.

Summary

A parallel decrease n the urinary calcium excretion and the Na/K ratio occurs in subjects with normal adrenal function after the cessation of a two-day treatment with chlorothiazide

In patients with Addison s disease on cortisol treatment no such decrease occurs. The Na/K ratio increases during treatment when mineralocorticoids are also administered but there is still a lack of response after treatment. A normal sodium-conserving mechanism after treatment evidently requires the presence of an adrenal gland capable of reacting with increased production of mineralosteroids.

The excretion of calcium behaves in many ways similarly to the Na/K ratio. From the data it appears that mineralosteroids are required for a decrease to occur after cessation of treatment, which seems to indicate that these steroids are in some way involved in the renal handling of calcium.

Acknowledgement

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Changes in the Urinary Excretion of Calcium after Intravenous Injection of Aldosterone

by

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In previous communications we reported on the marked decrease in the urinary calcium excretion which occurs after cessation of a two-day treatment with chlorothiazide (5, 6, 10). A superficial resemblance was observed between the behaviour of the urinary Na/K ratio and the calcium excretion. In patients with Addison's disease maintained with cortisol and in one case in which both cortisol and aldosterone were given such a response did not occur (6). The conclusion was drawn that the reaction seen in the Na/K ratio and calcium excretion was in some way dependent on the mineralosteroids and the hypothesis was put forward that it may be due to an activation of the renin-angiotensin-aldosterone mechanism, which is known to play a role in renal sodium conservation. In order to test this hypothesis some studies were carried out with aldosterone. The present report deals with the effect of a single injection of aldosterone on the urinary Na/K ratio and calcium excretion.

Material and Methods

Eleven tests were performed in 10 patients by giving a single intravenous injection of aldosterone. In addition, aldosterone injection was combined with spironolactone treatment in 6 instances.

The patients studied were mainly subjects with congenital heart disease (open duct or small atrial septal defect) without changes in renal or adrenal function. There were 6 women and 4 men of ages ranging from 16 to 32 years.

The tests were carried out as follows: The whole test period comprised 3 to 4 days. On these days urine was collected at hourly intervals from 8.00 a.m. to 2.00 p.m. In order to ensure that sufficient urine was passed, the patients were given 150 ml tap water to drink every hour starting at 7.00 a.m. On the test days the patients remained in a recumbent position during the period of urine collection. Starting the day before and throughout the test period the patients were given the ordinary hospital diet from which milk, cheese and fish were omitted.

In the first series of experiments the *first day* was a control, during which no treatment was given. On the *second day* 0.5 mg of *d*-aldosterone (Aldocorten[R] Ciba) was administered intravenously at 10.00 a.m. After urine collection was

concluded treatment with spironolactone (Aldactone A[R] Searle) was started. Spironolactone was administered orally in doses of 100 mg every 6th hour This treatment was continued throughout the third day and on the fourth day until urine collection was concluded. On the *fourth day* 0.5 mg of aldosterone was again administered at 10.00 a.m. Urine was not collected during the third day

I the second series of experiments no spironolactone treatment was given. After 2 or 3 control days during which urine was collected as described above, 1.0 mg of aldosterone was administered on the 3rd or 4th day

The urine samples were analysed for calcium, phosphorus, sodium, potassium and creatinine with the same methods as previously (11)

Treatment of the excretion data: The *excretion of calcium* was evaluated from the calcium/creatinine ratio (Ca/Cr) in order to take int account the filtration. The Ca/Cr ratio of each urine sample was calculated and the alue obtained for the first two hours (8.00 to 10.00 a.m.) was used as point of reference. The ratio of each urine sample collected after 10.00 a.m. (the time t which aldosterone was administered) was compared with this control level of the same day and expressed as ratio,

$$R = \frac{Ca_i/Cr}{Ca_o/Cr}$$

where indicates the control level. The changes in the ratio are shown for each individual case and each separat test day in figs. 1 t 10.

The *N/K ratio* was calculated for each urine sample and treated as described for calcium.

In order to evaluate the *effect of aldosterone* on the Ca/Cr ratio, the ratio R (see abov) of the urine samples after aldosterone injection (R_{Ald}) was substracted from the ratio R of the corresponding samples during the control day (R_C) The difference $R_C - R_{Ald}$ was then expressed as percentage of R_C (Table III) The *effect of spironolactone* was evaluated in the same way by subtracting R_{Ald} from the of the corresponding urine samples during spironolactone treatment, R_S, and expressing the difference $R_S - R_{Ald}$ as percentage of R_{Ald} (Table III)

I addition, the total calcium excretion in mg during each 4-hour period from 10.00 a.m. to 2.00 p.m. was recorded for each test day (T ble IV).

Results

Aldosterone brought about a decrease in the *Ca/Cr ratio* in 5 instances out of 11 tests (figs. 1 to 10 Table III) (cases 4 5 6 8, and in case 6 when re tested 6a) All primary data are compiled in Tables I and II In the cases mentioned the Ca/Cr level remained distinctly below that of the control day In some cases the maximal decrease from the control level amounted to 80—90 per cent. In cases 2 and 3 the response was regarded as negative, whereas in cases 1 and 7 there was a very slight decrease. In case 9 however an increase was observed which may have been due to some error in creatinine determination, since the excretion of creatinine during the control period of the aldosterone day was unexpectedly high The urinary *Na/K ratio* decreased from the control level in all instances, although the response was rather weak in case 1

Spironolactone treatment was given in 6 instances but only in one case (case 6) was the effect of aldosterone on the Na/K and Ca/Cr ratios clearly inhibited by the treatment (Figs. 1 to 11 Tables II and III) In 2 other instances (cases 4 and 5) the dose of spironolactone used was evidently insufficient to counteract the effect of aldosterone, since neither the Na/K ratio nor the Ca/Cr ratio increased as compared with the response to aldosterone alone In case 3 the Ca/Cr ratio did not respond to aldosterone but there was nevertheless an increase during spironolactone treatment parallel with an increase in the Na/K ratio In cases 1 and 2 the Ca/Cr ratio did not respond to either aldosterone or spironolactone and in case

1 the effect of these substances on the Na/K ratio was also rather slight.

The *excretion of calcium* during the four hours from 10.00 a.m. to 2.00 p.m. (Table IV) usually showed rather small variations, and owing to the small amounts usually excreted the evaluation of these changes is difficult. However in many cases a decrease was observed during the aldosterone period and in two instances again an increase during the spironolactone period (cases 3 and 6) *i.e.*, in those two cases in which the Ca/Cr ratio also increased. These observations seem to support those on the changes in the Ca/Cr ratio.

Discussion

Despite rather wide individual variations in the behaviour of the Ca/Cr ratio after aldosterone injection, a marked decrease was observed in 5 instances out of 11. Furthermore a similar response was observed twice in the same subject. To this number of "positive" tests may further be added those 2 (cases 4 and 5) in which there was an evident aldosterone effect on both the Na/K and Ca/Cr ratios which was not inhibited by the administration of spironolactone, thus making 7 "positive" tests out of 13. Such a high proportion of "positive" tests suggests a type of reaction which is not merely random. This view is further supported by the parallel increase in both the Na/K and Ca/Cr ratios during spironolactone treatment in 2 instances (cases 3 and 6) and by the changes, albeit small, induced by aldosterone and by spironolactone in the 4-hourly excretion of calcium. It may be significant that in those cases in which no response to spironolactone was seen there was either no reaction in the Na/K ratio or no response to aldosterone in the first place. In a previous study (6) a slight increase in calcium excretion during spironolactone treatment was also observed.

A certain parallelism has been reported to exist between the urinary excretion of sodium and of calcium (4, 11, 12) and a similar impression was gained in our studies with chlorothiazide (5, 6). There are, however, situations in which this does not hold true. The response in case 9, for instance, shows a dissociation of calcium and sodium excretion after aldosterone injection. But, on the other hand, the present series also includes examples of the parallel behaviour of the Ca/Cr and Na/K ratios.

Although the main response in the present cases was a decrease in the Ca/Cr ratio, *i.e.* a decrease in the calcium excretion, the possibility of other responses cannot be excluded. Glaubitt (3) recently reported an increase after aldosterone treatment in two cases. The reason for such inconsistencies may be complex. If calcium and sodium really compete for some common binding sites in the distal tubule, as has been suggested (11), then the type of response to aldosterone, and to spironolactone, for that matter, may depend, for instance, on the filtered load of the respective ions and the ratio between the loads, as well as on the dose of aldosterone or spironolactone. It is even possible that large doses of aldosterone act preferably on sodium reabsorption, whereas smaller doses affect both sodium and calcium reabsorption.

In the subjects tested, the morning level of the Na/Ca ratio (meq of sodium/

mg of calcium) varied from 0.25 to 6.0 but there was no correlation between this ratio and the type of response to aldosterone. It may be of some significance that in the cases that were given 0.5 mg of aldosterone a positive response usually included a fall in the Ca/Cr ratio below the morning control level, whereas a positive response to 1.0 mg did not include such a marked decrease. The

Table I. The hourly excretion of sodium, potassium, calcium and creatinine in the urine during the control day and after intravenous injection of 0.5 mg of aldosterone with and without concomitant treatment with spironolactone

Case No.		Control period Hours					Aldosterone Hours					Aldosterone+spironolact. Hours				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	Na	6.7	12.1	8.3	17.2	13.0	6.7	9.8	6.9	8.8	11.9	15.0	14.2	8.4	7.6	7.5
	K	1.1	1.4	2.7	1.2	4.2	1.3	2.7	3.4	3.8	7.7	3.5	4.8	5.3	2.5	1.3
	Ca	1.2	0.7	4.1	6.4	4.8	1.6	2.5	3.8	3.0	2.7	2.1	1.5	2.3	3.6	3.5
	Cr	63	23	50	86	85	72	89	42	66	43	115	60	43	63	45
2	Na	21.0	18.2	22.4	32.0	18.2	12.8	13.2	11.6	5.4	8.7	22.4	23.8	13.7	10.2	11.0
	K	13.5	9.1	10.5	16.0	12.4	5.5	4.9	6.1	4.2	7.6	5.6	5.3	5.1	4.8	4.5
	Ca	9.0	10.4	13.3	13.0	12.3	6.6	9.9	9.8	8.1	8.7	11.1	8.4	9.3	9.3	4.0
	Cr	75	65	63	45	39	67	33	35	48	33	70	17	45	30	35
3	Na	26.9	30.0	21.2	24.2	27.0	9.4	9.0	5.4	6.3	5.6	15.4	17.9	14.3	17.3	14.4
	K	12.3	9.8	5.6	4.1	1.9	7.3	4.5	6.2	5.3	4.2	6.7	3.1	3.9	3.5	2.4
	Ca	13.8	16.5	14.4	19.1	24.3	4.0	6.2	11.0	12.5	9.9	10.8	14.7	13.2	22.7	12.5
	Cr	63	60	32	33	45	21	36	26	32	19	64	25	23	43	9
4	Na	6.4	6.2	6.9	6.4	7.5	9.6	6.7	4.3	6.6	3.1	15.4	8.0	6.3	6.8	5.6
	K	5.6	3.5	2.8	3.4	5.4	4.8	2.2	3.0	3.8	3.4	5.8	4.2	5.4	4.9	3.3
	Ca	3.6	2.6	8.3	5.4	5.1	7.2	6.6	4.8	5.4	4.2	7.7	6.4	6.0	4.1	3.3
	Cr	74	23	26	26	26	48	33	32	27	17	55	40	49	38	37
5	Na	9.4	12.0	14.7	16.2	15.7	8.3	9.6	7.9	9.8	4.0	17.7	13.8	7.9	8.3	10.0
	K	8.1	4.0	4.9	5.2	6.8	3.5	3.8	5.9	8.2	6.3	6.7	4.3	4.3	5.3	4.5
	Ca	3.3	5.5	3.0	2.9	2.6	8.2	3.0	2.2	2.4	2.0	2.4	2.9	1.6	1.5	1.6
	Cr	86	80	62	46	39	69	70	67	84	60	18	91	74	67	67
6	Na	11.5	12.7	3.6	7.6	7.0	6.8	5.8	2.2	1.4	1.8	16.7	16.4	9.2	6.0	13.4
	K	9.3	6.5	1.5	4.3	4.3	4.3	3.3	4.7	5.1	7.6	8.0	6.6	3.9	4.6	5.9
	Ca	5.5	4.3	4.7	9.7	4.1	4.7	6.2	3.8	3.6	5.2	6.7	6.1	6.3	9.9	6.8
	Cr	85	80	50	[illegible]	40	54	60	50	40	50	85	90	30	80	50
6a	Na	8.8	7.9	6.3	7.5	8.9	8.6	5.3	2.8	2.4	1.6					
	K	7.5	6.0	3.7	4.9	6.4	8.4	4.2	7.0	8.1	11.5					
	Ca	8.8	12.3	24.0	3.0	8.0	12.1	8.1	10.1	7.3	3.1					
	Cr	83	100	60	60	140	24	32	38	26	12					

Excretion of Na and K as meq per hour that of Ca and creatinine (Cr) as mg per hour 0 = the control level of each separate day

l the effect of these substances on the Na/K ratio was also rather slight.

The *excretion of calcium* during the four hours from 10.00 a.m. to 2.00 p.m. (Table IV) usually showed rather small variations, and owing to the small amounts usually excreted the evaluation of these changes is difficult. However in many cases a decrease was observed during the aldosterone period and in two instances again an increase during the spironolactone period (cases 3 and 6) i.e., in those two cases in which the Ca/Cr ratio also increased. These observations seem to support those on the changes in the Ca/Cr ratio.

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Despite rather wide individual variations in the behaviour of the Ca/Cr ratio after aldosterone injection, a marked decrease was observed in 5 instances out of 11 Furthermore, a similar response was observed twice in the same subject. To this number of "positive" tests may further be added those 2 (cases 4 and 5) in which there was an evident aldosterone effect on both the Na/K and Ca/Cr ratios which was not inhibited by the administration of spironolactone, thus making 7 "positive" tests out of 13 Such a high proportion of "positive" tests suggests a type of reaction which is not merely random. This view is further supported by the parallel increase in both the N /K and Ca/Cr ratios during spironolactone treatment in 2 instances (cases 3 and 6) and by the changes, albeit small, induced by aldosterone and by spironolactone in the 4-hourly excretion of calcium. It may be significant that in those cases in which no response to spironolactone was seen there was either no reaction in the Na/K ratio or no response to aldosterone in the first place In a previous study (6 a slight increase in calcium excretion during spironolactone treatment was also observed.

A certain parallelism has been reported to exist between the urinary excretion of sodium and of calcium (4 11 12) and a similar impression was gained in our studies with chlorothiazide (5 6) There are, however situations in which this does not hold true. The response in case 9 for instance, shows a dissociation of calcium and sodium excretion after aldosterone injection. But, on the other hand, the present series also includes examples of the parallel behaviour of the Ca/Cr and Na/K ratios.

Although the main response in the present cases was a decrease in the Ca/Cr ratio, i.e a decrease in the calcium excretion, the possibility of other responses cannot be excluded. Glaubitt (3) recently reported an increase after aldosterone treatment in two cases. The reason for such inconsistencies may be complex. If calcium and sodium really compete for some common binding sites in the distal tubule, as has been suggested (11) then the type of response to aldosterone, and to spironolactone, for that matter may depend, for instance, on the filtered load of the respective ions and the ratio between the loads, as well as on the dose of aldosterone or spironolactone. It is even possible that large doses of aldosterone act preferably on sodium reabsorption, whereas smaller doses affect both sodium and calcium reabsorption.

In the subjects tested, the morning level of the N /Ca ratio (meq of sodium/

concluded treatment with spironolactone (Aldactone A[R] Searle) was started. Spironolactone was administered orally in doses of 100 mg every 6th hour. This treatment was continued throughout the third day and on the fourth day until urine collection was concluded. On the *fourth day* 0.5 mg of aldosterone was again administered at 10.00 a.m. Urine was not collected during the third day.

In the second series of experiments no spironolactone treatment was given. After 2 or 3 control days during which urine was collected as described above, 1.0 mg of aldosterone was administered on the 3rd or 4th day.

The urine samples were analysed for calcium, phosphorus, sodium, potassium and creatinine with the same methods as previously (11).

Treatment of the excretion data. The *excretion of calcium* was evaluated from the calcium/creatinine ratio (Ca/Cr) in order to take into account the filtration. The Ca/Cr ratio of each urine sample was calculated and the value obtained for the first two hours (8.00 to 10.00 a.m.) was used as point of reference. The ratio of each urine sample collected after 10.00 a.m. (the time at which aldosterone was administered) was compared with this control level of the same day and expressed as ratio,

$$R = \frac{Ca_0/Cr}{Ca_x/Cr}$$

where indicates the control level. The changes in the ratio are shown for each individual case and each separate test day in figs. 1 to 10.

The *Na/K ratio* was calculated for each urine sample and treated as described for calcium.

In order to evaluate the *effect of aldosterone* on the Ca/Cr ratio, the ratio R (see above) of the urine samples after aldosterone injection (R_{Ald}) was subtracted from the ratio R of the corresponding samples during the control day (R_C). The difference $R_C - R_{Ald}$ was then expressed as percentage of R_C (Table III). The *effect of spironolactone* was evaluated in the same way by subtracting R_{Ald} from that of the corresponding urine samples during spironolactone treatment, R_{Sp}, and expressing the difference R_{Sp} R_{Ald} as percentage of R_{Ald} (Table III).

In addition, the total calcium excretion in mg during each 4-hour period from 10.00 a.m. to 2.00 p.m. was recorded for each test day (Table IV).

Results

Aldosterone brought about a decrease in the *Ca/Cr ratio* in 5 instances out of 11 tests (figs. 1 to 10. Table III) (cases 4, 5, 6, 8, and in case 6 when re-tested, 6a). All primary data are compiled in Tables I and II. In the cases mentioned the Ca/Cr level remained distinctly below that of the control day. In some cases the maximal decrease from the control level amounted to 80—90 per cent. In cases 2 and 3 the response was regarded as negative, whereas in cases 1 and 7 there was a very slight decrease. In case 9 however an increase was observed, which may have been due to some error in creatinine determination, since the excretion of creatinine during the control period of the aldosterone day was unexpectedly high. The urinary *Na/K ratio* decreased from the control level in all instances, although the response was rather weak in case 1.

Spironolactone treatment was given in 6 instances but only in one case (case 6) was the effect of aldosterone on the Na/K and Ca/Cr ratios clearly inhibited by the treatment (Figs. 1 to 11. Tables II and III). In 2 other instances (cases 4 and 5) the dose of spironolactone used was evidently insufficient to counteract the effect of aldosterone, since neither the Na/K ratio nor the Ca/Cr ratio increased as compared with the response to aldosterone alone. In case 3 the Ca/Cr ratio did not respond to aldosterone but there was nevertheless an increase during spironolactone treatment parallel with an increase in the Na/K ratio. In cases 1 and 2 the Ca/Cr ratio did not respond to either aldosterone or spironolactone and in case

Table II. The hourly excretion of sodium, potassium, calcium and creatinine in the urine during the control day and after intravenous injection of 1.0 mg of aldosterone[a]

Case No.		Control period[b] Hours 0	1	2	3	4	Aldosterone Hours 0	1	2	3	4
7	Na	7.0	9.8	6.1	7.5	13.6	5.5	4.0	3.6	2.5	2.7
	K	2.3	1.5	1.4	3.2	5.0	8.6	1.0	2.5	6.6	4.2
	Ca	8.5	13.3	7.6	9.7	12.3	7.6	4.0	11.2	5.4	5.1
	Cr	70	36	32	23	37	59	18	40	18	32
8	Na	4.8	6.0	6.3	7.3	5.3	5.7	5.5	4.0	5.2	3.8
	K	5.1	8.3	2.9	5.7	2.5	4.6	1.4	3.8	5.5	9.7
	Ca	1.3	2.8	6.5	3.5	2.6	4.3	7.7	5.0	9.5	5.3
	Cr	66	*7	39	39	24	60	70	50	50	50
9	Na	5.9	8.5	8.3	7.7	9.9	5.3	6.2	3.1	1.2	0.9
	K	4.0	4.7	3.4	2.8	3.7	1.8	2.5	2.8	1.3	1.4
	Ca	7.7	6.8	6.8	7.2	6.0	8.0	5.8	6.5	4.7	3.2
	Cr	85	42	32	93	66	174	60	63	72	67
10	Na	11.8	4.5	16.1	9.3	6.0	11.2	18.4	8.0	8.0	3.3
	K	4.3	1.0	2.4	1.6	2.4	3.7	2.2	4.4	3.7	5.8
	Ca	14.3	15.7	30.7	16.2	14.9	13.8	21.4	15.5	13.9	10.4
	Cr	92	57	81	55	53	72	35	60	50	59

[a] Excretion of Na and K as meq per hour that of Ca and creatinine (Cr) as mg per hour 0 = the control level of each separate day

[b] Mean values for 2 control days in cases 7, 8 and 10 and for 3 control days in case 9.

Table III. Percentage change in the Ca/Cr ratio induced by aldosterone and spironolactone

Dose of aldo-sterone	Case No.	Aldosterone vs. control day Hours after injection of aldosterone 1	2	3	4	Spironolactone + aldosterone day vs. aldosterone day Hours after injection of aldosterone 1	2	3	4
0.5 mg	1	−22	− 6	−50	− 7	+8	−29	+53	+49
	2	+125	+39	−30	0	+4	−54	+15	−73
	3	− 17	+24	−12	+27	+286	+34	+34	+198
	4	0	−67	−47	−38	−33	−31	−40	−15
	5	−80	−77	−85	−84	−33	−43	−29	−35
	6	+45	−39	−63	−23	+28	+212	+50	+43
	6a	−50	−97	−31	0				
1.0 mg	7	−31	− 8	−34	−35				
	8	−76	−90	−63	−83				
	9	+15	+49	+50	+63				
	10	12	−43	−21	−57				

same is seen from Table III. In preliminary studies with aldosterone infusions we have observed that in some subjects, at least, smaller doses induce a decrease whereas larger doses may, in fact, bring about an increase in calcium excretion. It is also conceivable that if the mineralocorticoids are involved in the physiological renal conservation of calcium the responses reflecting such an action are observable only when the dosage is kept close to the physiological range. These aspects are being studied further.

In addition, there are in all probability many other factors capable of influencing the urinary calcium excretion and the response to aldosterone and spironolactone. In view of the events occurring after chlorothiazide treatment (5, 6, 10), angiotensin and the antidiuretic hormone (ADH) require especial consideration. Gantt *et al.* (2) recently reported that angiotensin induced a decrease in the calcium excretion and an increase in the serum calcium level. If the renin-angiotensin-aldosterone mechanism is involved in the response to chlorothiazide, as suggested (5, 10), angiotensin may modify the response in calcium excretion in some way and may also have some bearing on the elevation of the serum calcium level observed (5, 6, 10). Angiotensin alone is probably not responsible for the reaction in the urinary excretion of calcium, since there was a lack of response in patients with Addison's disease although in such patients the secretion of renin is probably not defective. But as is known with regard to sodium excretion, angiotensin seems to modify the response to aldosterone (1) in addition to stimulating the tubular reabsorption of sodium. ADH, on the other hand, has been shown to induce an increase in calcium excretion (7, 8) which would counteract the response observed in the antidiuretic phase after chlorothiazide treatment (5, 6, 10). Calcium evidently inhibits the inactivation of ADH in the kidney (9).

At present it is impossible to draw any far-reaching conclusions. But the results so far obtained indicate that in certain circumstances aldosterone is capable of decreasing the urinary excretion of calcium and that this response can

Table IV. The four-hourly excretion of calcium in the urine (mg) during the control day and after intravenous injection of aldosterone with and without spironolactone treatment (10.00 a.m. to 2.00 p.m.)

Case No.	1	2	3	4	5	6	6a
Control day	16.0	49.0	74.3	21.4	14.0	22.8	49.0
Aldosterone	12.0	36.5	39.6	21.0	9.6	18.8	26.6
Aldosterone + spironolactone	13.0	32.0	63.1	19.8	7.6	29.1	
Case No.	7	8	9	10			
Control days	41.9	15.2	26.8	75.3			
Aldosterone	23	27.7	22.7	61.7			

Two control days in cases 8 and 10, three control days in case 9.

probably be inhibited by spironolactone under appropriate conditions. Although being far from uniform the reaction was so prominent in several instances that it is difficult to find any other explanation. Mineralocorticoids may then play some role in the renal handling of calcium. Previous evidence suggests (6) that they are also in some way involved in the regulation of calcium excretion after chlorothiazide treatment. The problem is, however not solved by the present observations but requires further studies.

Summary

The effect of a single intravenous injection of aldosterone on the calcium/creatinine (Ca/Cr) and sodium/potassium (Na/K) ratios in the urine was studied in 10 subjects without adrenal or renal disturbances. In more than half the tests there was a decrease in both the Ca/Cr and Na/K ratios, and in one case a slight increase in the former The effect of aldosterone was inhibited by spironolactone in 2 cases out of 6, in the remaining cases either spironolactone was ineffective as regards the N /K ratio or there was primarily no response of the Ca/Cr ratio to aldosterone.

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The Effect of Corticotropin and Synthetic Angiotensin on the Glucose-6-phosphate and the Succinic Acid Dehydrogenases in the Adrenal Cortex of the Rat

By

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The activity of glucose-6-phosphate dehydrogenase (G-6-PD) in the adrenal gland (15,24) is low in the *zona glomerulosa* (ZG) and higher in the inner zones (3 4 17 18) G-6-PD activity is increased by stimulation with corticotropin (ACTH) in the rat (17 18) and in man (37) Succinic acid dehydrogenase activity (SAD) is low in the ZG and likewise reaches peak values in the inner zones (12, 13 14 22, 27 39) After stimulation with ACTH the activity of this enzyme does not increase significantly in the rat when measured either per mg of protein (13) or histochemically (27)

The importance of the hexose monophosphate pathway enzymes has been underlined by the discovery that reduced triphosphopyridine nucleotide (TPNH = NADPH) is required in the steroid hydr oxylation processes (16 26, 32, 38, 41) Clarification of the functional zonation within the adrenal cortex has been the objective of several studies in the past and more recently it has been shown that 21 and 11-hydroxylation and 3β-hydr oxysteroid dehydrogenation take place in both the ZG and the inner zones, whereas 17-hydroxylation occurs only in the inner zones and 18-hydroxylation only in the ZG (34 35 36) The stimulation of the hexose monophosphate pathway enzymes by ACTH (17) is in conformity with the hypothesis of Haynes and Berthet (20) according to which ACTH stimulates adrenal phosphorylase by way of the cyclic 3' 5-adenosine monophosphate, which results in increased production of glucose-6-phosphate and so in enhanced formation of the TPNH required for steroid hydroxylation.

The hormonal pattern after stimulation with ACTH as compared with that after angiotensin and renin treatment has been studied by several uthors (1 7 11 23 33 and others) ACTH has been shown mainly to stimulat the formation of the steroids derived from the inner zones but also somewhat the production of aldosterone. On the other hand, angiotensin seems mainly to stimulat aldoster one production but also to some extent

that of other steroids Some effects of angiotensin and renin on the ZG are by now well known (19 25 36, 40 and others) and it has been suggested that the changes induced by sodium restriction in many ways similar to those seen after angiotensin and renin treatment, are induced through the mediation of the renin mechanism (19 40) Cohen and Crawford (5 6) have recently shown that sodium depletion in rats induces a marked increase in the G-6-PD activity of the ZG a finding more recently confirmed by Marx *et al.* (28)

It thus seemed of interest to study the effect of angiotensin on the G-6-PD and SAD activity in the ZG since no infor mation was available at the time this study was commenced. Meanwhile Marx *et al.* (29) have reported an increase of G-6-PD activity after angiotensin treat ment.

Experimental

For series 1 to 3 female whit rats of the Sprague-Dowley strain weighing between 200 and 300 g were used and for series 4 and 5 male rats of the same strain weighing about 50 g at the start of the experiment. The animals were killed in ether narcosis by heart dissection.

Series 1 Fiv groups of 5 animals each were used. Tw untreated groups served as controls. Two groups were injected intraperitoneally with 100 µg of angiotensin II (Val-5-Hypertensin-II-amide, Hypertensin[R] Ciba) in saline solution 24 and 2 hours before sacrifice. It was calculated that the amount of sodium introduced in the form of saline was negligible as compared with the intake by w y of food, which contained 0.5 of sodium chloride One group was given tw injections of ACTH 6 units each, the same time intervals.

The glucose-6-phosphat dehydrogenase activ ty of he adrenal gland was determined hemically with commercial ki (TC W C, F Boehringer & S hne GmbH, Mannheim, Germany After the glands had been freed from surrounding connectiv tissue they were weighed on planchet with an automatic balance. The glands were then homogenized in Krebs-Ringer phosphate buffer in an ice bath with the aid of ultrasound. The homogenate was centrifuged and the enzyme activity determined from aliquots of the supernatant.

Series 2. (5-day experiments) Three groups of 5 animals each were used. One untreated group served as control. Another group was given tw daily intraperitoneal injections of 50 µg angiotensin in saline solution for 5 days. The third group received daily injections of 6 units of ACTH with prolonged action for 5 days. In series 2 t 5 one adrenal gland was used for histochemical studies, the other for routine histological studies.

Series 3. (2 to 3 week experiments) Three groups of 5 animals each were used, of which one untreated group served as control. Another group was given daily injections of 100 µg angiotensin i saline solution for 3 weeks. The third group was given daily injections of 6 units of ACTH with prolonged action for 2 weeks.

Series 4 (3 week experiments with young male rats). Two groups of 4 animals each were used. One untreated group served as control, the other group was given daily subcutaneous injections in the neck region of 100 µg angiotensin suspended in walnut oil for 3 weeks.

Series 5. (5 week experiments with young male rats). Two groups of 4 animals each were used and treated as in series 4 The angiotensin treat ment was continued for 5 weeks.

All the treated animals in series 2 to 5 were killed on the day following the last injection. The histochemical localization of G-6-PD and SAD was accomplished in 30 µm/thick fresh frozen sections. The G-6-PD was stained by the method of Hess *et al.* (21) as described by Pearse (31) and the SAD by modification (31) of the method of Nachlas *et al.* (30) using nitrophenyl substituted ditetrazole (Nitro-BT[R] Dajac Lab., Philadelphia, U S. A.)

Results

Chemical determination of G-6-PD activity ACTH increased the enzyme activity significantly The mean $\Delta OD_{340\,nm}$ mg^{-1}

mm $^{-1}$ 10^{3} increased from 0 850 in the controls to 1 10 in the treated animals (P<0.01) Angiotensin had no effect (mean 0 864)

Histochemical observations In the control rats *G-6-PD* activity was discernible in all zones but t was very low in the ZG It was mainly concentrated in the inner zones, especially in the outer *zona fasciculata*. In the ZG the enzyme activity seemed to be concentrated in a subcapsular layer about 1—2 cells thick and a broad, almost inactive zone extended from there to the border of the *zona fasciculata* (Fig 1 a) In the ZG of the animals treated with angiotensin the active layer was wider and the activity greater than in the controls. Slight changes were already visible after 5 days of treatment; they were more marked in series 3 and 4 and most prominent in series 5. The width of the ZG was nearly doubled and the enzyme activity now occupied the whole zone, leaving only a faintly visible

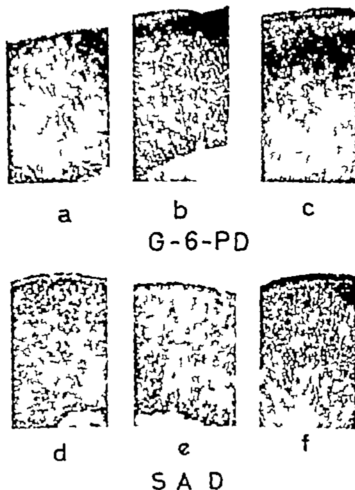


Fig 1 Photographs of the adrenal ortex of the test animals and d controls, b and c fter 3 weeks treatment, and f fter 5 weeks t eatment Note th intense stimulation f the enzyme activity of the zona glomerulosa in the 5 week animals and the intermediate response in the 3 week animal

inactive band 1–2 cells thick, at the *zona fasciculata* border The enzyme activity in the ZG appeared at some sites to exceed that of the outer fasciculata (Fig 1 c) When all series were compared it appeared that the activation of the ZG started from the subcapsular layer and extended towards the fasciculata border with increasing stimulation. ACTH induced an increase of the enzyme activity in the inner layers, mainly in the fasciculata. These changes were very slight in series 2 but clearly visible in series 3 The ZG appeared rather thin and it was apparent that the active fasciculata border was nearer the thin active subcapsular glomerulosa layer in the treated animals than in the controls.

The distribution of the *SAD* activity resembled that of G-6-PD In the controls the activity in the ZG was concentrated in a thin subcapsular layer (Fig 1 d) In the animals treated with *angiotensin* this layer was wider and the enzyme activity more marked than in the controls. In series 5 the SAD in the ZG was rather prominent and the histochemical appearance closely resembled that in the G-6-PD series (Fig 1 f) and again a thin inactive band separated the strongly activated ZG from the fasciculata.

ACTH also induced an increase of the SAD activity in the inner zones and, as in the case of G-6-PD the active fasciculata border was nearer the thin subcapsular glomerulosa layer in the treated animals.

Discussion

The hexose monophosphate pathway is an important source of TPNH in various endocrine tissues 8 9 10 In the adrenal gland TPNH is required for various hydroxylation processes (16, 26, 32, 38) and its formation in the inner zones is enhanced by stimulation with ACTH (17 18, 37) Renin and angiotensin are primarily involved in the stimulation of aldosterone production in the zona glomerulosa, where 18-hydroxylation seems to occur (34 35 36)

From this point of view the increase in G-6-PD activity in the ZG after stimulation with synthetic angiotensin II is of considerable interest. It was not evident in the short-term experiments when G-6-PD activity was measured by chemical means, whereas stimulation with ACTH brought about a profound increase in activity In histochemical studies some increase was observed after 5 days of treatment with angiotensin but it was marked only after 3 weeks. The findings are in conformity with the recent report of Marx *et al* (29) Although the widening of the ZG in routine histological sections was not very great it was nevertheless clearly observable. The dosage used in series 1 to 3 *i.e* 100 μg per animal per day corresponded roughly to the lowest dosage in the experiments of Marx *et al.* (29) With this dosage 50 μg/100 g daily these authors observed a slight increase in the width of the ZG in 50 g rats but the response was much more marked with higher dosage levels. In the present experiments the changes in the ZG were much more evident when a higher dosage was used 200 μg/100 g injected subcutaneously suspended in walnut-oil according to Marx *et al.* (29) for a period of 3 to 5 weeks The enzyme activity was more intense and the active layer wider

The effect of angiotensin II injections

on the ZG differs quantitatively from that produced by sodium restriction (5, 6, 28, 29) The adrenal glands of rats kept on a low sodium diet show a very marked widening and increase of the G-6-PD activity of the ZG within one to two weeks. The difference may be due to the short biological half life of injected angiotensin, in comparison with which a low sodium diet represents a constant stimulation On the other hand it has recently been shown that sodium depletion may induce adrenal changes even when the kidneys, the site of renin production, have been removed (2)

It would seem that an increase of the G-6-PD activity in the ZG may be indicative of increased activity of the hexose monophosphate pathway The evidence is so far very scanty however and Williams *et al* (42) have in contrast reported that *in vitro* angiotensin has no effect on the phosphorylase activity in bovine adrenal slices consisting mainly of glomerulosa cells.

The zonal distribution of SAD and G-6-PD was very similar In the control rats there was a thin subcapsular layer displaying enzyme activity and an inactive zone which separated the subcapsular layer from the fasciculata border In the animals treated for longer periods with angiotensin II the active layer containing SAD was wider and the enzyme activity more intense than in the control rats. This would possibly indicate that the most active layer as regard steroid formation in the ZG is the subcapsular layer and that factors which increase steroid production in this zone act mainly on this particular layer Whereas an increased G-6-PD activity may indicate activation of the hexose monophosphate shunt and increased steroid production the activation of SAD may merely indicate a more active over-all metabolism in these cells; but TPNH is also formed by way of the citric acid cycle.

It was also of interest to note that with increasing stimulation with angiotensin the enzyme activity in the ZG seemed to extend from the outer subcapsular layer towards the fasciculata border The relations between the enzyme activity and the morphological changes will be discussed more fully in a subsequent paper

Summary

The effect of angiotensin II injections on the glucose-6-phosphate and succinic acid dehydrogenase activity in the rat adrenal was studied histochemically A 3 to 5 week course of treatment, consisting of daily injections of about 50 to 200 μg angiotensin/100 g body weight, brought about a widening of the subcapsular layer containing both enzymes. In the most strongly stimulated animals the whole ZG showed a markedly increased enzyme activity

Two injections of ACTH brought about a significantly increased G-6-PD activity as measured chemically whereas angiotensin given in the same way was without effect. In animals treated with ACTH the enzyme activity increased in the inner zones and the zona glomerulosa grew thinner than in the controls.

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Two Different Pituitary-controlled Sulphation Mechanisms in the Rat[1)]

By

OTTO WEGELIUS AND CLAES FRIMAN

S^{35}-labelled sodium sulphate has been widely used as a tracer substance in experiments concerning the sulphation mechanism. High S^{35} incorporation into chondroitin sulphate (3 8) has been reported in the cartilage (10) and in organs containing sulphated mucopoly saccharides (5). Nearly all the radioactive sulphate administered is incorporated within 24 hours.

Sulphation is under endocrine control. Both factors which stimulate and factors which inhibit the sulphation mechanism have been indentified. Cortisone reduces the rate of sulphate fixation (4 14) and oestradiol reduces the incorporation of radioactive sulphate with the cartilage in the rat (16). Thyroxin increases the uptake in the cartilage in the rat (9) and in Harder's gland in the guinea pig (18). The pituitary gland is of particular interest. Hypophysectomy reduces the uptake (11) in the cartilage. The administration of somatotropic extracts stimulates the uptake in the cartilage *in vitro* not directly but via a sulphation factor present in the serum (1 17). On the other hand, thyrotropic pituitary extracts enhance the uptake of sulphate by the orbital glands in the guinea-pig (13).

This paper is a report on the effect of hypophysectomy on sulphate uptake by the rat orbital gland as compared with the known effect on the uptake in the cartilage.

The Problem

Experiments were planned so as to answer the following questions:

1) Is radioactive sulphate incorporated with sulphated mucopolysaccharides in the orbital glands — Harder's gland and the ventral lacrymal gland?

2) What is the effect of hypophysectomy on the uptake

3) How is the turnover of the radioactive compound influenced by removal of the pituitary gland

1) Part of the study was performed during fellowship at the Rockefeller Foundation

4) Is there any difference in the uptake between the glands and the cartilage?

5) What is the uptake of sulphate by the glands *in vitro*?

6) Is the uptake *in vitro* influenced by the serum factors of intact and/or hypophysectomized rats?

Material and Radioactive Technique

A hundred and forty-eigh young rats of the Wistar train were used in the experiments. Seventy-tw of them were hypophysectomized t the Hormone Assay Laboratory Inc. Chicago, Ill., U.S.A. E ery batch of opera ed rats was ma ched w th an equal batch of intact controls of the same age After every experiment it was checked that the removal of the anterior lobe of the pituitary gland had been complete. Four operated animals were injected during 4 successiv day with the bovine growth hormone preparation Somar A, Lot 916, total dose 50 μg in saline Four operated animals served as controls. On av erage the somatotropin-treated animals gained 8 g the controls only 1 g in 4 days.

A er sacrifice he glands were carefully dissected out, weighed and treated for the determination of radioactivity as previously described (15) The pancreatic gland and sample of costal cartilage were treated in the same w y Radioactivity was measured with mica end window Geiger Muller counter

Experiments and Methods

No I Sulphate is incorporated in the ventral lacrymal gland of the guinea-pig with a compound containing glucosamine and an unidentified hexuronic acid (5) In order to ascertain whether sulphate wa synthesized into a mucopolysacchar ide n the same way n our animals the following determinations were made:

A correlation between the hexosamine values and the incorporation of radioactive sulphate in Harder s gland the ventral lacrymal gland and the retrobulbar connective tissue in guinea-pigs was made

Five guinea-pigs were injected intra peritoneally with 360 μc $Na_2S^{35}O_4$ Twenty four hours after administration of the radioactive sulphate the animals were sacrificed and the tissues were dissected pooled and homogenized In the acetone dried homogenates the content of hexosamine and the radioactivity were deter mined. The remains of the homogenates were digested with papain by the method of Buddecke (6) The digested tissues were centrifuged filtrated and dialysed against running water for 46 hours. The dialysate was first precipitated with 3 vol. absolute ethanol, pH 10 at +4° C for 22 hours and then with 3 vol. ethanol pH 2.5 at +4 C for 22 hours. The precipitates from the different pooled tissues were analysed for hexosamine by the method of Elson and Morgan (12) and for S^{35} radioactivity (15)

The mean values for the tissues of all animals are shown n Fig 1 It is seen that after purification there is a clear-cut rise in the values for hexosamine and radioactivity Harder's gland and the retrobulbar connective tissue show relative ly higher hexosamine values as compar ed with the values for radioactivity than the ventral lacrymal gland This may be attributable to a higher proportion of unsulphated polysaccharides and/or protein-bound or dialysable sulphate disappearing during purification.

The same determinations were made in Harder's gland and the ventral lacrymal glands of 10 rats 24 hours after intraperitoneal administration of 200 μc $Na_2S^{35}O$ It was found that the homogenates contained 2 4 μg/mg of hexosamine and that the values for radioactivity were high. The radioactive sulphated compound

was undialysable after digestion with papain.

No. II Four hypophysectomized rats (mean weight 60 g, age 36 days, one week after operation) and 5 control animals (mean weight 121 g age 36 days) were injected intraperitoneally with 100 μc $Na_2S^{35}O_4$ 24 hours before sacrifice. The results are listed in Table I

The uptake of radioactive sulphate in the glands of the hypophysectomized animals was three to four times the uptake in the glands of the unoperated controls ($P<0.001$)

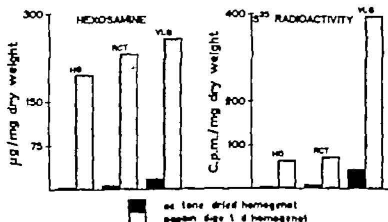
No III. In order to check the results the experiment was repeated with the difference that radioactivity was calculated against body weight. Every animal was given 100 μc $Na_2S^{35}O$ /100 g of body weight. Nine hypophysectomized rats (mean weight 80 g, age 55 days

Table I The effect of hypophysectomy upon the uptake of radioactive sodium sulphate ($Na_2S^{35}O_4$) by Harder' gland and the ventral lacrymal gland. The dose of radioactivity was 100 μc S^{35} per animal.

Group	Number of animals	Harder gland (cpm/100 mg)	Ventral lacrymal gland (cpm/100 mg)
		57	44
		58	57
Intact controls	5	52	77
		86	61
		68	99
		64±13.49	68±22.11
Hypophysectomized animals	4	200	168
		309	249
		234	222
		221	188
		241±47.44	207±35.91
		P<0.001	P<0.001

Fig. 1

The content of hexosamine and S^{35} radioactivity in acetone-dried homogenate and in papain-digested homogenate of Harder's gland (HG), the ventral lacrymal gland (VLG) and the retrobulbar connective tissue (RCT) of the guinea pig



two weeks after operation) and 9 intact controls (mean weight 151 g age 55 days) were used The results are shown in Table II

Even so the uptake in the glands was *high in the hypophysectomized animals*, or about twice the uptake in the controls ($P<0.001$)

No IV The effect of TSH administration on the uptake of radioactive sulphate after hypophysectomy was studied. Eighteen operated animals and 10 intact controls (mean weight 125 g age 45 days) were used. Eight hypophysectomized rats (mean weight 69 g age about 45 days, one week after operation) were injected with saline 5 hypophysectomized rats (mean weight 71 g age 45 days, one week after operation) were injected intraperitoneally with 2.5 U.S P u. of TSH (Thytropar Armour Laboratories Ill U.S.A.) and 5 hypophysectomized rats (mean weight 67 g age 45 days one week after operation) were injected with 5 U S P u of the same TSH preparation

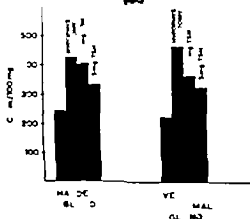


Fig 2
The effect of hypophysectomy and additional administration upon the sulphate uptake by Harder gland and the ventral lacrymal gland

Table II. The effect of hypophysectomy upon the uptake of radioactive sodium sulphate ($Na_2S^{35}O_4$) by Harder gland and the ventral lacrymal gland. The dose of radioactivity was 100 μc per 100 g body weight.

Group	Number of animals	Harder gland (cpm/100 mg)	Ventral lacrymal gland (cpm/100 mg)
		108	120
		139	115
		98	116
Intact controls	9	98	98
		85	100
		93	115
		88	98
		96	98
		93	104
		100 ± 16.09	105 ± 12.00
		203	229
		240	239
Hypophysectomized animals	9	212	177
		130	264
		174	181
		163	224
		298	170
		215	155
		311	131
		216 ± 59.83	196 ± 40.44
		$P<0.001$	$P<0.001$

All animals received 100 μc/100 g of body weight of $Na_2S^{35}O_4$ 24 hours before sacrifice.

The results are shown in Fig 2. The uptake of radioactive sulphate in the glands of the hypophysectomized animals again differed significantly from the uptake in the intact controls ($P<0.001$) The TSH administration reduced the uptake although not significantly

No. V The next step was to find out the rate of turnover of sulphate in the orbital and pancreatic glands and to compare the uptake and turnover in

the glands with that in the cartilage. Twenty-two hypophysectomized rats and 23 controls were injected with 100 μc/100 g of body weight of $Na_2S^{35}O_4$. Three groups comprising 5 operated animals and 5 controls were sacrificed respectively 8 hours, 24 hours and 48 hours after injection. A group of 4+4 animals was sacrificed 72 hours, and a group of 3+4 animals 96 hours, after injection.

The results are seen in Figs. 3—6 which show the turnover in Harder's gland and in the ventral lacrymal gland. Ninety-six hours after the administration of S^{35} the radioactivity in these glands was still significantly higher in the operated animals than in the normal controls. In the pancreatic gland the values for radioactivity differ significantly only at 24 hours.

The *reverse picture* was obtained in the cartilage, the hypophysectomized animals exhibiting a low and the normal animals a high uptake. This difference in the uptake of sulphate between the glands and the cartilage is striking.

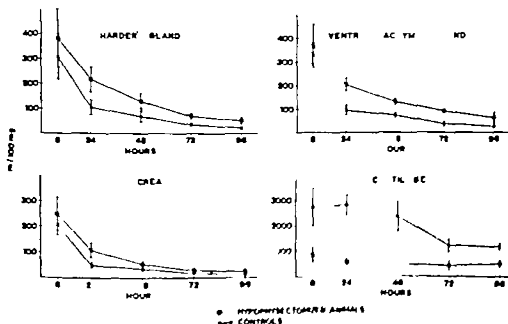
No. VI. In order to find out whether there is any serum factor in normal animals which depresses the uptake or in hypophysectomized animals which stimulates it, experiments with sera were made *in vivo* and *in vitro*.

In vivo

Five normal rats were injected with normal rat serum, five rats with serum

Figs. 3—6.

THE UPTAKE AND TURNOVER OF RADIOACTIVE SULPHATE IN HARDER'S GLAND, THE VENTRAL LACRYMAL GLAND AND THE PANCREATIC GLAND AND THE UPTAKE AND TURNOVER IN THE COSTAL CARTILAGE IN INTACT AND HYPOPHYSECTOMIZED RATS. NOTE THE HIGH AND REVERSE UPTAKE IN THE CARTILAGE AS COMPARED WITH THE UPTAKE IN THE GLANDS.



from hypophysectomized animals and 10 rats with saline. No difference in the uptake was demonstrable between the groups the counts being all of the same level (see Table III)

In vitro

Five normal rats aged 20 weeks and 5 hypophysectomized rats, aged 15.5 weeks, were used eight weeks after hypophysectomy. Only Harder's gland was dissected out under sterile conditions, and half of the gland was placed in a test tube for incubation.

The medium was prepared as follows. *To Eagle's solution 199* which served as a basic medium, radioactive S^{35} labelled sodium sulphate (in 0.04 per cent carrier solution in physiological saline phosphate buffer solution) was added, giving a concentration of 30 μc per cc. Of this solution 0.5 cc was used in each test tube (15 μc). A volume of *0.25 ml of Eagle's solution* was added to 10 tubes, 0.25 ml of freshly prepared serum from hypophysectomized rats was added to another 10 tubes, and 0.25 ml of freshly prepared serum from normal rats was added to 20 tubes.

After placing the samples of Harder's gland into prewarmed tubes, these were incubated for 4 hours at 37° C. Subsequently the tissue samples were washed quickly in physiological saline, weighed and placed in dialysing bags. Dialysis was performed overnight against 4 litres of water and later against 4 litres of water twice changed.

After dialysis the samples were ashed and the radioactivity determined in the same way [illegible] the *in vivo* experiments.

Uptake and incorporation of sulphate into the various components of Harder's gland took place both *in vitro* and *in vivo*. The *in vitro* experiment did not reveal any differences between the groups (Table III)

Table III. The influence of serum from hypophysectomized rats upon the uptake of radioactive sodium sulphate ($Na_2S^{35}O_4$) by Harder's gland, tested in vivo and in vitro.

IN VIVO

Group	Number of animals	Harder's gland (cpm/100 mg)
Controls	10	111
Normal serum controls	5	103
HYX serum	5	100

IN VITRO

Group	Number of test tubes	Harder's gland (cpm/100 mg)
Normal glands in normal serum	10	4490
Normal glands in Eagle	10	4640
HYX glands in HYX serum	10	4270
HYX glands in normal serum	10	4130

Summary of the Experimental Results and Conclusions

Harder's gland and the ventral lacrymal gland incorporate sulphate into a mucopolysaccharide like compound. After hypo-

physectomy the uptake increases significantly In the pancreatic gland the uptake shows the same pattern as in the glands just mentioned. After hypophysectomy the uptake in the glands is the reverse of the uptake in the cartilage. The uptake shows a significant increase in the glands and a significant decrease in the cartilage. After hypophysectomy the turnover is slower than in normal animals.

Harder's gland is capable of incorporating sulphate *in vitro*. Serum from normal and hypophysectomized animals did not influence the incorporation.

Discussion

The uptake of S^{35}-labelled sodium sulphate by Harder's gland and the ventral lacrymal gland in the guinea pig is stimulated by thyrotropic pituitary extracts (13) and has been introduced as an assay method for ophthalmotropic activity in TSH preparations (20) The influence of other hormones, *e g* somatotropin, on this uptake has been studied. Somatotropin had no stimulating effect (18)

Sulphate is incorporated into both the connective tissue stroma of the glands and into the gland cells, as shown by autoradiography (19) The sulphated component of the glands is a mucopolysaccharide (5 present investigation) The compound stimulating the glands to increased uptake is pituitary in origin and present in thyrotropic extracts.

In conjunction with somatotropic extracts the sulphation factor of Salmon and Doughaday (17) thoroughly investigated by Almqvist (2) has a stimulating effect on the chondroitin sulphate metabolism in the cartilage

After hypophysectomy there is a marked decrease in the uptake of sulphate in the cartilage, and a marked increase in the uptake in the glands. The pituitary origin of the sulphation factor is obvious in the cartilage metabolism. If the same factor were effective in the sulphation mechanism of the mucopolysaccharides throughout the organism, the uptake by the glands in question ought to be reduced after hypophysectomy This is not the case, however Two different ways of effecting peripheral sulphation have to be taken into account in order to explain the above results. It seems clear that in glands of epithelial origin sulphation is not controlled in the same way as in the cartilage, which is a mesenchymal tissue.

The question arises as to whether the pituitary has an inhibitory effect on the glands, too This point cannot be settled on the basis of the present study The most tenable explanation is, perhaps, that the hypothalamus is responsible for the stimulation of the sulphation of the glands after removal of the pituitary gland. Hypothalamus is known to have the capacity of producing thyrotropic and thyrotropin-releasing compounds (7) The *in vitro* studies where serum of hypophysectomized animals did not influence the incorporation argue against this assumption, however

Summary

Experiments with hypophysectomized rats and S^{35}-labelled sodium sulphate as a tracer substance showed conclusively that the removal of the pituitary gland influences the incorporation of sulphate in completely different ways in the

orbital glands and in the cartilage. After hypophysectomy Harder's gland and the ventral lacrymal gland take up and incorporate significantly more sulphate than they do in normal animals, whereas the incorporation significantly decreases in the cartilage. Certain experimental results seem to suggest that sulphate is incorporated into a mucopolysaccharide like compound, but probably not into chondroitin sulphate as in the cartilage. *In vitro* studies yielded no evidence of the existence of a circulating stimulating factor in the serum of hypophysectomized animals. This negative finding is discussed. The conclusion is drawn that the sulphation mechanisms for chondroitin sulphate and other sulphated polysaccharides or mucoproteins are not controlled in the same way. Two different endocrinely controlled sulphation mechanisms are present in the rat, one in tissues of epithelial, the other in tissues of mesenchymal origin.

Acknowledgements

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Accumulation of Connective Tissue Ground Substance in the Heart of the Dwarf Mouse

By

OTTO WEGELIUS

Dwarfism in the house-mouse was first described by Snell (1929) The conclusion was drawn by both him and other authors that the causative factor is a deficient pair of genes giving rise to a lesion of the pituitary gland. The latter lacks eosinophil cells and the capacity to produce somatotropin (6, 8, 10 12) Decreased thyroid function was early observed histologically (12, 14) and later this find ng was confirmed by the deter mination of PBI and the uptake of radioiodine (15) Furthermore, the pituitary origin of the thyroid hypofunction has been proved by timulation w th thyrotropic pituitary extracts, histologically observable activation (14) and increased uptake of radioiodine in the thyroid (15)

Observations on the cytology of the pituitary gland ha e been consistent with these findings. The adenohypophysis of the dwarf mouse lacks thyrotropic cells (4 16)

The dwarf mouse is in a state of pituitary myxoedema. Not only somatotropin but also thyrotropin and thyroxine, are lacking in its metabolism. Nature here offers a laboratory animal with partial selective hypophysectomy

The peripheral effect of thyrotropin on the connective tissue is not yet fully understood. This hormone has been regarded as a stimulator of the synthesis of the mucopolysaccharides of the ground substance. Since this factor is lacking, the mucopolysaccharide content ought to be low in the tissues of the dwarf mouse. By co trast, the content of these components ought to be high if thyroxine deficiency is the only factor of significance i the development of myxoedema.

This study was undertaken in order to compare the mucopolysaccharide content n th muscle tissue of dwarfs and normal animals of the same litter

Material and Method

The study was performed in three series, using the same methods on 20 dwarf mice and 21 normal animals of the same litters of the strain maintained at the Institute of Human Genetics, University of Copenhagen. The entire heart and skeletal muscle from the thigh were investigated. The hexosamine content was determined by the Elson-Morgan method. Statistical analysis was performed by Student's t-test.

Results

The hexosamine values are shown in Table I. For the skeletal muscle they were higher in the dwarf mouse group, than the values in the normal group. The content of hexosamine in the heart was significantly higher in the dwarf mouse group than in the normal animals.

Table I. The hexosamine content in skeletal and heart muscle of normal and dwarf mice

	Number of animals	Skeletal muscle	Heart muscle
Dwarf mice	20	1.75 ± 0.71	2.39 ± 0.41
Normal mice	21	1.39 ± 0.41	1.64 ± 0.25
		P < 0.025	P < 0.0005

The mean values of hexosamine expressed in µg per mg dry tissue ± standard deviation in skeletal and heart muscle of mice.

Discussion

It is believed that the synthesis of mucopolysaccharides in the connective tissue cells is stimulated by thyrotropin and inhibited by thyroxine. Schiller, Slover and Dorfman (1962) were able to show that the content of hyaluronic acid increased and the content of chondroitin sulphate decreased in the skin of the rat in primary myxoedema. This was due to absence of thyroxine and not to an increased TSH effect. Carlsöö and Ludvig (1957) showed that ground substance is accumulated in the human skin in both primary and pituitary myxoedema as a result of the absence of thyroxine and consequently not as a result of a TSH effect.

On the other hand it has been conclusively shown that a similar mucinous myxoedema develops in laboratory animals in the retrobulbar region after injection of thyrotropic extract (1, 9). Malignant exophthalmos and localized myxoedema occur in human beings simultaneously with an excess of thyroxine. It is obvious that pituitary factors play a dominant part in the development of any mucinous oedema of this type.

It is difficult to understand the conflicting results in these two sets of carefully performed investigations. Mucinous oedema is not caused by the hormone that theoretically only stimulates the thyroid, but it may be caused by the exophthalmos-producing substance and perhaps by the long-acting thyroid stimulator. Consequently the composition of the hypophyseal (hypothalamic) incretion must play an essential part.

An accumulation of hexosamine was observed in the heart of the dwarf mouse. This substance is a good indicator of the quantitative occurrence of mucinous substance in the heart muscle (2). In the skin (3) and skeletal muscle higher values for hexosamine were noted in dwarf mice than in normal animals of the same litter. It seems highly probable that mucinous oedema develops as a result of thyroxine deficiency alone.

It should be taken into account that in different environments the reaction of the connective tissue to various hor-

mones may differ both quantitatively and/or qualitatively This has previously been emphasized (11) and results pointing in this direction, relating to the sulphation of mucopolysaccharides, will be reported in a subsequent paper

Summary

Owing to a pituitary lesion, dwarf house mice lack the capacity to synthesize STH and TSH. These pituitary hormones are regarded as having a stimulating effect on the synthesis of mucopolysaccharides in the connective tissue. Thyroxine deficiency also leads to accumulation of mucinous substances. Significantly higher hexosamine values — the latter being an indicator of the content of mucopolysaccharides in the tissue — in the heart of dwarf mice as compared with normal animals of the same litter seem to indicate that thyroxine deficiency is the only factor of significance in the development of mucinous tissue oedema in connexion with pituitary myxoedema.

Acknowledgement

This study was aided by grant from the Sigrid Jusélius Foundation.

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It is believed that the synthesis of mucopolysaccharides in the connective tissue cells is stimulated by thyrotropin and inhibited by thyroxine. Schiller Slover and Dorfman (1962) were able to show that the content of hyaluronic acid increased and the content of chondroitin sulphate decreased in the skin of the rat in primary myxoedema. This was due to absence of thyroxine and not to an increased TSH effect. Gabrilove and Ludvig (1957) showed that ground substance is accumulated in the human skin in both primary and pituitary myxoedema as a result of the absence of thyroxine, and consequently not as a result of a TSH effect.

On the other hand, it has been conclusively shown that a similar mucinous myxoedema develops in laboratory animals in the retrobulbar region after injection of thyrotropic extract (1 9) Malignant exophthalmos and localized myxoedema occur in human beings simultaneously with an excess of thyroxine It is obvious that pituitary factors play a dominant part in the development of any mucinous oedema of this type.

It is difficult to understand the conflicting results in these two sets of carefully performed investigations. Mucinous oedema is not caused by the hormone that theoretically only stimulates the thyroid, but it may be caused by the exophthalmos-producing substance and perhaps by the long-acting thyroid stimulator Consequently the composition of the hypophyseal (hypothalamic) incretion must play an essential part

An accumulation of hexosamine was observed in the heart of the dwarf mouse This substance is a good indicator of the quantitative occurrence of mucinous substance in the heart muscle (2) In the skin (3) and skeletal muscle higher values for hexosamine were noted in dwarf mice than in normal animals of the same litter It seems highly probable that mucinous oedema develops as a result of thyroxine deficiency alone.

It should be taken into account that in different environments the reaction of the connective tissue to various hor

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The Hydroxyproline and Hexosamine Content in Human Myocardium at Different Ages

By

Otto Wegelius and Johan von Knorring

The chemical structure of collagen changes with increasing age (2 8 12) By contrast, it has not been possible to prove that there is a spontaneous quantitative increase in the fibrillar component of the connective tissue due to age alone. Fibrosis of the human myocardium is a common autopsy finding This deposition of collagen seems mainly to be secondary to a primary lesion of the coronary arteries or to inflammation of the myocardium. On careful microscopical examination such a process has been detected at as much as 10 per cent of routine autopsies (3 4 9)

There is no evidence of specific age changes taking place in the ground substance of the connective tissue e in the chemical composition and internal quantitative relationships of the acid mucopolysaccharides. Certain results obtained analytical studies of the myocardium seem to indicate that the total content of acid mucopolysaccharides decreases with age (5) Consequently a drop in the ratio ground substance/collagen would be a sign of ageing

We have studied disseminated fibrosis in human hearts without demonstrable coronary lesions. Speculating about the cause of the fibrosis, we posed the question as to whether the latter may be due to ageing

Material and Method

In connexion with autopsies[1] specimens were taken from 39 macroscopically normal hearts. The cause of death was in most cases an accident, in some cases poisoning, status epilepticus or subarachnoid haemorrhage. The age varied between one day and 60 years.

From each heart three specimens were taken from corresponding sites, from the middle of the left and right ventricular walls and from the same level of the septum. From each specimen

[1] We thank Professor Unto Uotila, M. D., Head of the Department of Forensic Medicine University of Helsinki, for the opportunity of obtaining material from his institute.

samples free from endocardium and pericardium were weighed immediately prior to hydroxyproline and hexosamine determination. I addition, samples were kept for histological examination. The interval between death and utopsy varied from one to five days. The specimens taken for chemical investigation were kept at −20 C until determinations were made.

Hydroxyproline was determined by the method of Neuman and Logan (12) and hexosamine by that of Elson and Morgan (6) The histological preparations were fixed in neutral formalin and 4 per cent basic lead acetate, sectioned at 5 µm and stained with haematoxylin-eosin and toluidine blue.

Results

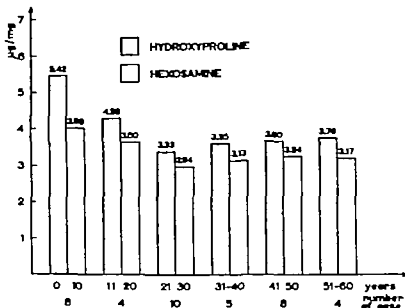
Fig 1 is a diagrammatic representation of the mean hydroxyproline and hexosamine content in each age group. All three determinations for each heart have been taken into account.

The lowest hydroxyproline and hexosamine values were noted in the age group 21−30 years. In the group 0−10 years the hydroxyproline values were higher than in the other groups — 5 42 µg/mg against 4.26 µg/mg in the group 11−20 years

The hydroxyproline and hexosamine content of the different heart regions in relation to age are shown in Fig 2. The age group 51−60 years excepted, the hydroxyproline content was highest in the right ventricle irrespective of age. In the youngest age group, in particular very high values were noted in this region In the septum and left ventricle, too the hydroxyproline values were high in the group 0−10 years. The subsequent age groups exhibited no marked differences in regard of hydroxyproline content in these regions.

Fig 1.

The content of hexosamine and hydroxyproline in the human heart at different ages.



In regard of the hexosamine content in the different heart regions no marked variations relating to advancing age were demonstrable. The hexosamine level varied less in the different heart regions than the hydroxyproline level. Nonetheless, similar age curves were obtained for the hydroxyproline and hexosamine content in the whole heart. The highest values were noted in the age group 0—10 years then the levels dropped in the subsequent two groups, the lowest values being obtained in the group 21—30 years. Subsequently somewhat higher values were noted, and the levels remained more even

The group 0—10 years excepted, the ratio hexosamine/hydroxyproline in the whole heart remained more or less the same, irrespective of age. No sex difference was observable. All hearts showed normal histological pictures.

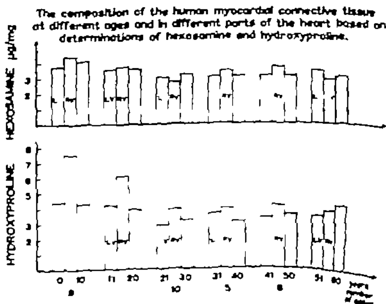
Heart weight in relation to content of connective tissue

Of a total of 32 normal hearts, 15 weighed less and 17 more than 300 g The mean content of hydroxyproline in the smaller hearts was 4.46 μg/mg of dry tissue against 3.51 μg/mg in the hearts weighing more than 300 g The hexosamine content was 3.51 μg/mg and 3.11 μg/mg of dry weight, respectively in the two groups.

Summarizing the results of these observations it may be stated that in normal histologically controlled human hearts the collagen and hexosamine content undergoes no significant changes correlated with age. An exception is constituted by the first ten years of life, during which the collagen level appears to be higher than later in life. No sex difference is observable.

Fig. 2

The composition of the human myocardial connective tissue at different ages and in different parts of the heart based on determinations of hexosamine and hydroxyproline.



Discussion

In regard of the collagen content in the myocardium of normal hearts, similar results have been obtained by other investigators (1 10 11 13) No age linked variations have been observed. Table I is a list of studies concerning the hydroxyproline content; the values obtained are indicated. It appears that no fibrous degeneration of the myocardium is demonstrable with increasing age by the available methods for quantitative chemical determination of collagen by hydroxyproline analysis. The high values in the youngest age group seem to be due to a more rapid development of the stroma than of the contractile elements during growth The higher content in small hearts, also observed by Montfort (11) and in the wall of the right ventricle (13) is attributable to functional causes. Increased contractibility yields a relatively larger number of muscle fibres.

Table I. Earlier reports on the collagen content in normal human myocardium.

Author	Number of cases	Collagen in per cent of dry tiss.	Influence of age	Sex differences
Blumgart *et al* 1940	55	4.80[1]	none	none
Oken *et al.* 1957	43	5.90 right ent	increasing up to 10 years	none
Lieves *et al* 1960	68		none	none
Montfort *et al* 1962	105	4 49	none	none

[1] Average figures calculated from values given by the authors

The hexosamine content in the heart muscle which may be regarded as a relatively good indicator of the mucopolysaccharide content (5) showed no significant differences between the groups. Clausen (5) reported that, with increasing age, hexosamine and uronic acid showed a tendency to drop, while the acid mucopolysaccharides and the ratio hexosamine/hydroxyproline decreased significantly Perhaps his results on a large series give a clearer picture in this respect. Our results do not confirm those of Clausen, although they do not definitely contradict them either

Summary

The content of hydroxyproline and hexosamine in the normal histologically controlled human myocardium did not show any significant changes with age. The highest values, indicating a proportionally stronger connective tissue stroma, were noted in the age group 0—10 years. The same observation i.e more connective tissue, less contractile elements, was made in small hearts. It is concluded that the connective tissue does not change quantitatively with age.

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Severe Disseminated Myocardial Fibrosis in Man without Coronary Involvement

The Result of an Autoimmune Process?

By

Otto Wegelius and Johan von Knorring

Fibrosis of the myocardium is a relatively common necropsy finding. Studies based on the determination of hydroxyproline lend no support to the hypothesis that the collagen content in the heart muscle increases with advancing age (11, 44, 55).

As far as we know to-day, it is the result of many severe myocardial lesions. The myocardial lesions here concerned have a varying aetiology, and the nomenclature is confusing.

Atherosclerosis of the coronary vessels causing both localized and wide-spread fibrotic changes is the commonest of the lesions in question, and it constitutes a homogenous group. Disorders with a primary lesion of the small vessels of the complex circulatory system of the myocardium are less well understood. The same may be said of the diseases of a probably autoimmune origin which are believed to affect the muscle fibres and the interstitial components, the connective tissue (36).

Efforts to detect microscopically visible differences in myocardial fibrosis of different origin have failed. However, some promising results with fluorescent antibodies have been reported (34). The best way of attacking these difficult problems is the experimental approach, *i.e.* to produce lesions in the myocardium in laboratory animals. Myocardial biopsies may throw some light on the problem. Therefore, hearts with disseminated myocardial fibrosis but without any signs of coronary or/and valvular involvement were collected at necropsies. The clinical data were studied, and all necropsy specimens were histologically reinvestigated in order to throw light on the etiology by collecting information about concurrent lesions in other organs.

Material and Methods

During the five-year period 1957–1961 necropsies were performed in 62.5 per cent of the fatal cases from the ward of internal medicine. The total number of necropsies was 1358. Dure

Table III. Clinical data and histological findings in seven cases of heart death with disseminated fibrosis of the myocardium without coronary involvement or diseases of other organs.

Age	Sex	Clinical diagnosis	Duration of the disease	Blood pressure ECG	Heart weight	Autopsy findings
54	F	Congestive heart failure pulmonary embolism.	many years	105/80, Low voltage, auric.fibrill., transit. bundle branch block.	500 g	The muscle fibres are fragmented, the interfibrillar spaces are large. Perivascular fibrosis. Abundant mast cells. Foci of mononuclear cells, predominantly lymphocytes.
60	F	Congestive heart failure, nodular goitre	6 years	109/90, auric. fibrill., left bundle branch block.	440 g	Nodular goitre. Fragmented muscle fibres in the myocardium. Perivascular fibrosis. Abundant mast cells. Large foci of predominantly lymphocytes.
57	F	Congesth heart failure, thyrotoxicosis? nodular goitre	8 years	150/90 Low voltage, ventr extra-syst.	5[illegible]0 g	Nodular goitre. No signs of hyperactive thyroid gland. The muscle fibres seem atrophic, fragmented. Large foci of lymphocytes. Few mast cells.
54	F	Congestiv heart failure chronic myocarditis.	10 months	120/75, Low voltage.	450 g	The interfibrillar spaces are large. Patches of fibrosis. Some mast cells. Scattered lymphocytes.
48	M	Sudden heart death	?	?		Disseminated fibrosis of the myocardium. Marked fibrosis also in patches. Lymphocytes scattered in the tissue. Some mast cells.
50	M	Sudden heart death	?	?	?	Disseminated fibrosis of the myocardium. Verrucous *endocarditis. Foci of* granulocytes and lymphocytes. Abundant mast cells.
57	M	Congestive heart failure	4 years	140/80 Low voltage, auric. fibrill., right bundle branch block.	810 g	Disseminated fibrosis of the myocardium. Perivascular fibrosis.

in other organs or renal damage, did not differ from the hearts of the patients listed in Tables II and III

The histological changes in the hearts of the patients listed in Tables II and III can be summarized as follows: No detectable changes of the capillaries. The small vessels exhibited a thickened adventitia. The sarcoplasm appeared to be swollen. Lipofuscin was present within the muscle cells. The cross-striation of the muscle fibres was strongly decreased or absent. The interstitial connective tissue showed the greatest changes, "interstitial myocarditis In some sites fibroblasts were abundant. The amount of collagen was highly increased. Among the free cells, lymphocytes and plasma cells predominated. Some granulocytes, especially neutrophils, were seen. The free cells were mostly located around the small vessels and between the muscle fibres.

Ten cases of advanced coronary sclerosis with secondary myocardial fibrosis served as histological controls. This material was treated in the same way as was described in the foregoing Histologically the test groups and the control group differed in regard of the occurrence of free cells in the myocardium. In the test groups, foci of mononuclear cells mainly lymphocytes, were scattered in the tissue. In the control group this was a very uncommon finding Lymphocytes were seen but never in the same number as in th test groups.

Discussion

Apart from coronary atherosclerosis, many other factors have been reported as causes of an isolated myocardial disease resulting in fibrosis of the myocardium. Isolated myocarditis forms a heterogenous group. Many clinicians and pathologists have discussed this problem (6, 7 9 10 23 29 48, 56) In particular an infectious aetiology has been suggested for many cases in this group.

De la Chapelle and Kossman (18) reported a frequency of myocardial lesions of 10 per cent among all necropsies. Kline and Saphir (38) found myocardial involvement in 8 per cent in a series of 2652 necropsies. In a group consisting of only bronchopneumonias, the frequency of myocardial lesions rose to 38.8 per cent (49) when the myocardium was carefully investigated. In a series of 1402 cases of myocarditis, Gore and Saphir (27) were able to establish that only 10 per cent were of rheumatic origin, and that a correct clinical diagnosis had been made in only 30 per cent.

Virus infections in particular have been discussed as a possible causative factor in isolated myocarditis (1 20, 19) This possibility was pointed out, for instance, by von Bonsdorff (6) Isolated myocarditis has also been reported in connexion with toxoplasmosis and tuberculosis (56) In Chagas' disease a relationship between the causative agent, *Trypanosoma cruzi*, and the isolated involvement of the myocardium has been especially well documented (22, 32, 39)

Even though an infection discovered or undiscovered may play a part in the development of isolated myocarditis, as was first established by Fiedler (1899) all cases cannot be explained on this basis. Today Fiedler' myocarditis, isolated myocarditis, idiopathic myocarditis and pernicious myocarditis are different names for a syndrome with severe myo-

cardial damage of unknown aetiology. Clinically the disorder is characterized by a constantly downhill course with heart failure and a low blood pressure, changes in the ECG etc. terminating in death. Pathologically disseminated myocardial fibrosis without any special histological signs in a dilated heart is found. The course of the disorder has been described as hyperacute, simulating coronary occlusion (26), acute (1, 50) in combination with some familial disposition (4, 45) or more chronic (2, 5, 28, 41, 50). Although a great deal of experience has accumulated in the course of years thanks to these observations and the findings in extensive series (13, 38, 52), the aetiology still remains to be clarified.

In some cases isolated myocarditis has been reported in conjunction with some other disease. These observations increase our possibilities of solving the problem. The clinical course and the pathological findings are very similar to those seen in Fiedler's myocarditis.

In cases of malnutrition and alcoholism where the possibility of *beri-beri* exists, isolated myocarditis resembling Fiedler's myocarditis has been observed (53).

Amyloidosis has been a concurrent phenomenon in some instances (12).

Myocardial lesions similar to Fiedler's myocarditis have been found in patients suffering from mesenchymal disorders. These so-called *collagen diseases* are characterized by high gamma globulin values in the blood and specific changes of the mesenchyme. Isolated myocarditis has been reported in disseminated lupus erythematosus (8) and in scleroderma (19, 31, 43, 54). Severe myocardial damage is also known to occur in cases of dermatomyositis and polyarteritis nodosa (56). In a case of Fiedler's myocarditis, fibrinoid degeneration in the vascular walls of the spleen was observed (40).

In addition *sarcoidosis* has been suggested as a possible cause of the myocardial lesions in Fiedler's myocarditis (42), but the interrelationship seems very uncertain (29).

In *pregnancy toxaemia* (56) and *toxaemia post partum* (7, 47) myocardial damage of equal severity to that seen in Fiedler's myocarditis may develop.

The classification of the present material was based solely upon the necropsy findings of disseminated myocardial fibrosis without visible involvement of the coronary arteries. The distribution of the cases seen in Tables I—III is in good accordance with what is known about different groups of isolated myocarditis.

The histological findings in the hearts in this otherwise heterogeneous group are similar. Notwithstanding small differences in appearance — the changes being granulomatous, more disseminated or perivascular — the myocardial fibrosis could not be subdivided into different groups on the basis of the routine histological methods employed. Of special interest is the population of free cells in the tissues. Mononuclear cells, predominantly lymphocytes, were seen in almost every case. The occurrence of lymphocytes and/or plasma cells has repeatedly been reported by others, too (1, 2, 4, 39, 42, 50, 52).

Pronounced myocardial fibrosis cannot be conclusively explained as the result of a burned-out process — a reparative phenomenon. The clinical course of the

disorder with a steady deterioration must be due to a persistent process. The round-cell infiltrations speak in favour of this, too and t seems most probable that the active process is localiz ed to these foci.

Similar foci are a common finding in autoimmune diseases. They can be found in Hashimoto's thyroiditis with antithyroglobulin antibodies (13) The same antibodies have been found in malignant exophthalmos (50) where the retrobulbar tissues show infiltration of lymphocytes and fibrosis.

Experimental studies have yielded several examples of autoimmune processes with lymphocytic and plasmocytic infiltrations.

Is there any evidence of an autoimmune process being the causative mechanism in myocardial disorders In animal experiments, autoantibodies against heart muscle tissue ha e been produced (24 25 33, 45 55) Heart muscle antisera alone and especially in combination with dead streptococci or in a previously beri-beri damaged heart are able to produce changes resembling myocarditis. With homogenate of rabbit heart muscle iso-antibodies can be produced in the rabbit, and the organ specificity of the antibodies has been established (24 25,37)

Autoantibodies against heart muscle tissue have also been observed in man. In Chaga s disease Jaffé (32 was able to demonstrate such autoantibodies against both muscle tissue and trypanosomes. Antimyocardial autoantibodies in man have been detected in myocarditis, after myocardial infarction the post-myocardial infarction syndrome (16, I after mitral valvulotomy and n the post-commissurotomy syndrome (35)

Whether these auto-antibodies are capable of producing myocardial damage under certain circumstances we do not know but the possibility exists. In human rheumatic hearts, Kaplan and Dallenbach (36) using the immunofluorescence method, observed gamma globulin deposits between the muscle fibres, in the muscle sarcoplasm and in the endothe lium of the heart capillaries. It is too early however to say whether these deposits represent specific autoantibodies.

The collagen diseases are mostly ex plained on the basis of autoimmune processes. Bardawil *et al* (3) were able to produce antibodies against nucleoproteins from tissue cells — a very interesting finding especially with disseminated lupus erythematosus in mind. The foci of round cells in the myocardium seen in collagen diseases and the cell infiltrations in the myocardium seen in cases of completely unknown aetiology point in the direction of a similar pathogenesis. It seems possible that an autoimmune process, starting from an initial muscle tissue damage of varying aetiology leads to the severe changes seen, for instance, in the present cases. A similar explanation was suggested by Kaplan (34)

The lymphocyte and the plasma cell — cells from th same source, the thymus — have been connected with antibody formation. Their presence in the myocardium would be accounted for by the occurrence of n immunological process

If the lymphocyte is a stem cell with an additional capacity of developing int a fibroblast with collagen-forming qualities its presence in a fibrotic tissue is expl i d 21

Conclusions

A series of cases with disseminated fibrotic changes in the myocardium without coronary involvement, selected from a routine necropsy material, constituted a group with features strongly resembling those reported in cases of isolated myocarditis.

By ordinary histological methods it is impossible at autopsy to find any clue to the aetiology or to detect any real differences in the histological picture in the different cases.

In addition to fibrotic changes, similar histological changes were observed in the collagen disease group and in the group with myocardial involvement alone. Foci of mononuclear cells were a common finding. Lymphocytes predominated.

Considering the various data and discoveries available from the field of autoimmunization, the possibility of an autoimmune process being a causative factor in isolated myocarditis exists.

Summary

Twenty-four cases of disseminated myocardial fibrosis without coronary lesions selected from 1358 necropsies during a five year period are reported. In seven cases the aetiology was uncertain, in four cases there was a concurrent collagen disease, amyloidosis was present in three cases, tuberculosis in one, colitis ulcerosa in one, and one case exhibited pregnancy toxaemia. The findings in these cases were in good agreement with those made by others in isolated myocarditis. In three cases rheumatic changes in other organs, and in four cases renal lesions, were observed.

The group of collagen diseases and the cases of unknown aetiology were investigated histologically by routine methods. No differences between the individual cases were detectable, and foci of mononuclear cells were found in all. Lymphocytes predominated. It is assumed that the process in the heart muscle was still active at the time of death, and that the foci of round cells were the sites of this activity.

The collagen diseases are mostly explained on an autoimmunological basis. Lymphocytes are found in the tissues in disorders known to be autoimmunological. The similarity of the group with an unknown aetiology and the group of mesenchymal disorders, with respect to the occurrence of interstitial free cells is regarded as evidence of an autoimmunological process being secondary to a primary lesion of the myocardium of varying aetiology. This possibility is discussed in the light of recent results in the field of immunology and cytology.

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The Protein Coating of Red Cells in Experimental Immunohaemolytic Anaemia

By

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Great diagnostic importance is attached to the demonstration by Coombs test of a protein coating on the red cells in immunohaemolytic anaemia (2, 5) but the phenomenon itself is also of interest and is still the subject of speculation and research. In most cases with warm antibodies, the coating has been found to contain 7 S-gamma-globulin, the typical antibody fraction of serum (6, 7) The cold antibodies are 19 S-macroglobulins. Complement is readily adsorbed on the red cell — macroglobulin complex, and is in many cases probably the major cause of a positive Coombs reaction (6) However cases of immunohaemolytic anaemia have been observed in which warm reacting non-gamma-globulin proteins on the red cells constitute all or a part of the coating substance (21) In addition, cells damaged by antibodies or various other substances may adsorb plasma proteins onto their surfaces (3 8, 9 16, 22) In many cases, complement or fractions of complement have been found in the coating substance (10 17 20)

Numerous experimental studies on immunohaemolytic anaemia have been published. In most of the experiments reported, heterologous anti-red cell sera have been injected (23) but studies with iso-antibodies have also been made (26) A few workers have studied the protein coating of red cells after injection Muratore *et al.* (15) injected anti-red cell serum from rabbits into guinea pigs, and on the fourth day found a homologous (recipient species) coating on the erythrocytes, simultaneously with the development of severe anaemia. In their experiments, Lille-Szyszkowicz and Chojnacka (13) also used guinea pigs and rabbit immune serum. Coombs test, performed with anti-guinea-pig serum, became positive two or three days after the injection, and simultaneously autoagglutination of red cells, and erythrophagocytosis were

observed Samaille *et al* (18 19) inject ed anti-dog red cell serum from rabbits into dogs, and tested the dog red cells for a protein coating The cells were immediately coated with rabbit protein, and 5 hours later with dog protein. When Coombs test was performed with anti-dog serum, the reaction gradually strengthened during the first 24 hours. Young *et al* (26) studying the haemolytic effect of iso-antibodies in dogs, found that the agglutinating effect of dog anti-A, and anti E sera was aug mented by normal serum components.

Material and Methods

Rats of the Sprague-Dowley strain, weighing 150—300 grams, were used as test animals. *Haemolytic immune sera* were produced in 7 guinea-pigs by means of repeated intraperitoneal and subcutaneous injections of washed rat erythrocytes. The doses varied between 0.1 ml of 20 per cent suspension, and 1 ml of 40 per cent suspension, and the number of injections ranged from 14 to 20. Blood was drawn by heart puncture, and the sera separated and inactivated at 56°C for two hours with and without addition of pooled lyophilized guinea-pig complement (Difco Laboratories) using 2 per cent suspension of pooled rat red cells. Three sera were used for the experiments:

Serum	Complete lysis	End-point lysis	Agglutination
A	1/96	1/768	1/1576
B	1/768	1/3072	1/3072
C	1/96	1/384	1/3072

Serum A was obtained from single animal. Sera B and C were mixtures of sera from number of immunized guinea-pigs.

Anti-serum sera (*Coombs' sera*) Two rabbits were immunized with rat serum, and two with guinea-pig serum, the same time-table being followed. The animals were first treated with 6 intravenous injections of serum, given in doses of 1 ml on alternat days. The treatment was then continued with injections once week for 6 weeks. Of these injections, three consisted of 2 ml serum with complete Freund adjuvant and were given subcutaneously; the others were given intraperitoneally One week after the last (intraperitoneal) injection, the animals were bled. The sera were inactivated, absorbed three times with rat red cells, and titrated by the capillary tube technique using serial dilutions of rat serum and guinea-pig serum. The sera used for the tests had the following precipitin titres:

	Antig	
	Rat serum	Guinea-pig serum
Anti-rat serum	1/2,000	negative
Anti-guinea-pig serum	1/200	1/2,000

The anti-guinea-pig serum reacted weakly to rat serum; this unspecific effect was tested several times with agar plates. At times weak precipitation line was observable, but not always. The anti-rat serum did not react to guinea-pig serum on agar plates.

The conglutinin titres were determined by method II A of Coombs *et al.* (4), with slight modifications.

The production of immuno-conglutinin in rats was stimulated by course of 4 injections of *E. coli* vaccine (lot I) administered every third day the same treatment being repeated after rest period of 18 days. In addition, some of the rats were given two booster doses of more concentrated vaccine (lot II) 18 days after the second course. The vaccine had been prepared by the method of Abruzzo and Christian (1) in the following way: The strain of *E. coli* was first maintained for months, with weekly transfers, in medium containing only inorganic salts and glucose. The organisms were then killed by addition of formalin (0.4 per cent), and after washing, suspensions were made to the original volumes with normal saline. The optical densities of the suspensions were determined at 650 nanometres with Beckman model B spectrophotometer The optical density of lot I was 0.405, and that of lot II 0.635.

Results

I Red cell destruction in vivo

Most of the rats were given 0.3 ml anti-red cell serum per 100 grams of body weight intravenously After the injection haemolysis proceeded in two phases, the first began within a few minutes, the red cell count dropping 1—2 millions per mm in about two hours. Most of this initial haemolysis occurred within the first 30 minutes. During the second and slower phase a further red cell drop of 1—3 millions was noted. The slow haemolytic phase lasted for several days, and the lowest red cell count was usually recorded on the third or fifth day

In two group experiments, anti-red cell serum was injected into previously untreated rats, and into rats pretreated with *coli* vaccine to raise the conglutinin titre. All the rats were given anti red cell serum intravenously Both groups consisted of 6 rats, 3 of them with serum conglutinin titres above 1 4 and 3 with a titre of 1 4 or less. Unfortunately no very high conglutinin titres were obtained. Table I shows that the haemolysis was more pronounced in the previously untreated animals. In the group which had been given the stronger immun serum B, 2 of the rats without immuno-conglutinin died whereas no deaths occurred among the rats with titres above 1 4 There was no over lap, but the material is too small f r statistical evaluation.

II The anti-protein (Coombs) reaction

Red cells from a rat which had been injected with anti-red cell guinea-pig serum days previously were washed 4 times.

Table I. The red cell drop in rats injected with anti-red cell serum.

Exp. No.	Serum injected	Conglutinin titre	Maximum red cell drop, millions/mm
36	B	1:4	4 4
38	B	1:4	Died
39	B	1:4	Died
22	B	1 16	3.0
29	B	1:64	2.8
35	B	1:32	2.2
32	C	1:4	3.1
33	C	1:4	2.3
37	C	1 4	2.4
24	C	1:8	1.6
27	C	1 16	2.2
42	C	1 16	1 7

A 2 per cent suspension was prepared, and tested with serial dilutions of anti-rat serum and anti-guinea-pig serum. One drop of red cell suspension and one drop of diluted serum were incubated for 30 minutes at room temperature, centrifuged for one minute at 800 r p.m. and exa mined macro- and microscopically after careful shaking Definite agglutination was seen at the following dilutions.

	1/10	1/20	1/40	1/80	1/160	1/320	1/640
Anti-rat serum	2+	2+	1+	1+	—	—	—
Anti-guinea-pig serum	—	±	1+	2+	2+	1+	±

Thus the red cells were found to be coated with both rat protein and guinea pig protein. It is interesting to note that a definite prozone phenomenon occurred with anti-guinea pig serum, but not with anti-rat serum. For further Coombs tests, nti-rat serum was diluted 1:20

and anti-guinea-pig serum 1:100. No agglutination of normal rat cells was at any time observable at these serum concentrations with the reading technique described.

In 24 experiments, in which rats injected with guinea-pig antibodies to rat cells were used, a study was made of the time course of the two Coombs tests. The first blood specimens were obtained 5—15 minutes after the injection. The washed red cells of this first sample were always strongly agglutinated by anti-guinea-pig serum. With anti rat serum, the test was negative 15 minutes after the injection in 11 cases, and weakly positive in 13. In all the 11 cases which were negative at 15 minutes, the anti-rat serum reaction subsequently became positive; this usually occurred within 30—120 minutes. However, in 3 cases the reaction was still negative 2 hours after injection, but positive the following day. In these cases, the reaction was not recorded between 2 and 24 hours. The anti-guinea pig reaction was as a rule already maximal (3+) 15 minutes after injection, whereas the anti-rat reaction gradually increased in strength, to become maximal 1—2 days later. Both reactions then decreased, to become negative within 3—10 days. This decrease was usually parallel for both reactions, but in some cases the anti-rat serum reaction disappeared sooner. In general, the anti-rat reaction was somewhat weaker than the anti-guinea pig reaction.

The intensity of the anti-guinea pig reaction of washed cells varied very little from case to case whereas the anti rat reaction showed marked individual variation.

Large quantities of blood were withdrawn from some of the rats. If these are excluded, there remained 18 survivors, which were followed for a week or more. In addition, 2 animals died from haemolysis. On comparison of the strength of the anti rat serum reaction with the severity of the anaemia which developed after the injection, some correlation is discernible. In 8 cases, the Coombs reaction with anti rat serum never became stronger than 1+. The average red cell drop in these rats was 2.7 millions per cu. mm. In 10 rats, there was a strong (2—3+) reaction, the corresponding average red cell drop being 3.7 millions. However, the limited material makes it impossible to ascribe any statistical significance to the difference. The red cells of both the animals that died from haemolysis exhibited a strong anti-rat serum reaction. No correlation existed between the time required to develop a definite rat protein coating of the red cells, and the severity of the haemolytic reaction *in vivo*. Neither was there any correlation between the conglutinin titres and the Coombs reactions.

III. *In vitro tests*

Three rats were bled 3—24 hours after the injection of anti-red cell serum, and red cell eluates were prepared by the method of Weiner (25). According to Coombs test, the red cells were coated with both rat and guinea-pig protein before elution. The eluates were incubated with normal rat erythrocytes for 2 hours at 37°C, and Coombs tests made after washing. All the eluates were found to produce heavy coating of the red cells with guinea-pig protein. With anti-rat

protein serum, the most concentrated eluate gave a weak positive indirect Coombs test; it had been prepared by the addition of only half a volume of saline to the red cells eluted. The other eluates, which were 4—5 times as dilute, gave negative indirect Coombs tests with anti-rat serum.

It was hoped that the nature of the homologous coating substance could be elucidated by immunoelectrophoresis, but no distinct precipitation lines were obtained on the agar plates. When the eluates were tested against the anti-serum sera by immuno-diffusion on agar plates, no precipitation lines were obtained around two of the eluates. Very thin lines could be seen close to the most concentrated eluate towards both anti-rat serum and anti-guinea-pig serum, although the lines were partly obscured by a non-specific circular precipitation around the eluate. The same eluate, diluted 1 10, caused less nonspecific precipitation, but the specific (?) lines were completely absent.

Small blood samples were drawn from several rats 5—15 minutes after the injection of anti-red cell guinea-pig serum. At that time, cells were coated with guinea-pig protein, but Coombs test with anti-rat serum was still negative. The cells were washed and treated with rat serum and serum fractions. Incubation of a 5 per cent suspension of cells with undiluted fresh serum for 1—2 hours at 37°C rendered the cells faintly Coombs-positive when tested with anti-rat serum. However the reaction was sometimes so weak as to be recorded ±. If the samples were taken somewhat later when the anti-rat Coombs reaction had already become slightly positive an augmentation of the reaction after incubation was noted. No homologous coating was demonstrable either after incubation at lower temperatures, or with diluted rat serum. Normal serum inactivated at 56°C for 30 minutes had no effect, nor did inactivated serum of coli vaccinated rats (conglutinin titre 1.8). The result was also negative if small amounts of fresh serum were added to the inactivated serum. Finally different crude fractions of rat serum complement were tested, i.e. the soluble and the insoluble fraction obtained by dialysis against hypotonic phosphate buffer zymosan treated serum, and ammonia treated serum (11) but no rat protein could be demonstrated on the cells after incubation.

Discussion

It has long been known that in most experiments a given amount of haemolytic antibody destroys more red cells *in vivo* than *in vitro* (23). This phenomenon might be attributable to destruction *in vivo* of red cells coated and damaged by "sublytic" amounts of antibody. Mechanical forces, phagocytosis, and the splenic circulation in particular are factors acting in the living organism which are not present in the test tube (24). The continuous production of complement by the living organism is certainly important, but *in vivo* other humoral or tissue factors might contribute to the destruction of red cells after the injection of red cell antibodies. Lee *et al.* (12) produced anti-red cell serum against mouse erythrocytes in rabbits, and fractionated the

sera by starch block electrophoresis. On testing the various fractions, they found a discrepancy between the lytic effect *in vitro* and *in vivo*.

In agreement with others (13, 15, 18, 19), we have demonstrated the existence of a homologous factor on the red cells after the injection of heterologous antibody to red cells. This substance is demonstrable by means of antiserum to homologous serum, but it is produced by the recipient organism, and might thus also be termed "autologous". This factor appeared within a few hours after injection, sometimes in less than 15 minutes. Consequently it cannot be an anti-antibody in Milgrom's sense (14). It has been suggested that the autologous coating substance is a means of protection against haemolysis (19). Conversely, it has been thought that this substance participates in the slow phase of haemolysis (13, 15). In our experiments, a strong anti-recipient Coombs reaction usually concurred with a severe haemolytic anaemia. The autologous substance thus seems to contribute to the destruction of red cells.

We have not been able to determine the nature of the autologous substance. A prozone phenomenon was found in connexion with the anti-guinea pig Coombs reaction, but not with the anti-rat reaction. In human immunohaemolytic disease, Dacie (6) found a prozone when the red cells were coated with gamma globulin whereas no prozone was present if the coating consisted of other proteins. From this it may be inferred that the autologous coating substance in our experiments was probably not gamma globulin.

The heterologous antibody injected was easily recovered by elution of red cells, but most of the autologous substance was lost. If a small quantity of red cells coated with antibody injected into an animal was incubated with rather large amounts of undiluted fresh rat serum, there appeared a weak coating with rat protein, although it was appreciably weaker than the autologous coating on the red cells obtained from the animal a few hours later. It seems likely that a part of the autologous coating consists of one or several components of complement. Unfortunately it was not possible to verify this by incubation with fractions of fresh rat serum. In any case, it is believed that complement factors constitute one component of the autologous coating, but that other factors are probably added *in vivo*. In view of the interaction between conglutinin and complement (4), it did not seem too far fetched to assume the presence of conglutinin on the red cells, but the findings do not support this assumption. After pretreatment of rats with killed *E. coli* bacilli, there was no correlation between the conglutinin titres and the autologous coating. However, the conglutinin titres were not very high. It was noted with surprise that the pretreated rats seemed to become somewhat less anaemic than the normal rats after the injection of anti-red cell serum, but the material is too small to allow of definite conclusions.

Summary

Immunohaemolytic anaemia was produced in rats by the injection of anti-rat erythrocyte serum obtained from

immunized guinea pigs. The protein coating of the red cells was studied by means of Coombs' tests, using anti-serum sera from rabbits. Guinea pig protein could be demonstrated on the red cells within a few minutes of the injection. The Coombs reaction with anti rat serum became positive after a short period, usually within two hours, and gradually became stronger during the first 24 hours. The anti-rat Coombs reaction was stronger in rats with severe anaemia than in animals which developed a mild haemolytic anaemia. No correlation existed between the conglutinin titre and the autologous coating of the red cells, but animals pretreated with a view to raising the conglutinin titre seemed to be more resistant to immunohaemolysis than did normal rats. If rat red cells sensitized *in vitro* with heterologous antibody were incubated with homologous serum, a faintly positive anti-rat Coombs reaction occurred, but it was much weaker than the reaction obtained on testing red cells from animals suffering from immunohaemolytic anaemia.

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From the Minerva Foundation Institute for Medical Research, Helsingfors, Finland.

Immunoelectrophoresis of Human Saliva

By

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Although the macromolecular components of human saliva have been studied by means of electrophoresis (4–6, 7–11, 12, 18, 19–20, 21–24, 26–32, 34–35), analytical ultracentrifugation (26) and column chromatography (25), the results have been variable and somewhat inconsistent. Immunochemical techniques, notably immunoelectrophoresis with anti-human serum antiserum, have also been used (5–8, 9–22). These studies indicate the presence of several plasma proteins in saliva. Rabbit anti-human saliva serum has also been employed, but the resolution in previous immunoelectrophoretic studies (5–10) has been inferior to that obtained in conventional zone electrophoresis (6, 26–34).

We have used rabbit anti-human saliva serum in earlier studies (14–28) mainly to investigate the occurrence of common antigens in different body fluids. In the present study rabbit anti-saliva serum has been employed to develop immunoelectrophoretic patterns of whole, parotid and submaxillary saliva. Some of the components in the resulting patterns were identified.

Materials and Methods

Saliva. Whole saliva was collected by having healthy laboratory personnel chew paraffin. The collection period was half an hour. The samples were immediately cleared by centrifugation at +4 C, and were then treated separately or pooled, depending on the purpose. After centrifugation, the saliva was concentrated twentyfold by ultrafiltration at + 4°C. The concentrate was used for the immunizations and electrophoretic fractionations.

Parotid saliva was obtained with modified Lashley cups (3). Submaxillary saliva was secured by placing a suction cup over the orifice of the submaxillary duct. The secretion of saliva was stimulated by instilling dilute acetic acid into the mouth. These two secretions were then concentrated by ultrafiltration in the cold. Before electrophoresis, all the samples were dialyzed at +4 C against the electrophoresis buffer.

The protein content of the concentrated saliva samples was assayed by the method of Lowry

et al. (23) and was found to be bout 1 per cent. Clinton Chemtrol® bovine standard serum was used to produce the standard curve.

Cysteine hydrochloride or mercaptoethanol in phosphate buffer was sometimes added to the saliva samples prior to immunoelectrophoresis, the final concentration of each being 0.1 M (16)

Mucus clot. This material was prepared by freezing and thawing the saliva. The clot was washed three times with physiological saline and finally dissolved in 2.5 per cent sodium bicarbonate.

Amylase About 100 ml saliva was centrifuged and passed through glass wool. It was then fractionated on Sephadex G-25 and calcium phosphate columns, essentially according to the method of Millin and Smith (25) Amylase activity was determined as described by Smith and Stocker (30). The three amylase-active peaks emerging from the calcium phosphate fractionation step were first ultrafiltered to volume of 1.5 ml, then equilibrated against sodium phosphate buffer of pH 7 4 and ionic strength 0.1 and finally subjected to Pevikon electrophoresis as described by Gräsbeck *et al.* (15) In this procedure all the three amylase peaks emerging from the preceding step had the same mobility only one 280 mμ light-absorbing, amylase-active peak being observed in each case.

Antisera. 1 Anti-saliva serum. Two rabbits were each given 20 mg of salivary protein (Lowry) with the complete Freund adjuvant divided into two subcutaneous injections administered t one week's interval. Two weeks after the last subcutaneous injection the rabbits were given 4.5 mg of salivary protein absorbed onto alumina gel and divided into 5 intravenous injections administered every second day Two weeks after the last injection the rabbits were found to have precipitin titre exceeding 1 1064 as titrated against the original saliva pool diluted 1 10. They were bled by heart puncture and the sera were stored in the deepfreeze. One month after the end of the first immunization course booster course was initiated. It consisted of 3 intravenous injections totalling 3 mg of protein per rabbit. Further blood was taken by heart puncture 2 weeks after the completion of the booster course. These sera were pooled with the earlier sera.

Other rabbits were immunized according to other schedules, and high precipitin titres were obtained, but the resulting anti-sera gave fewer lines in immunoelectrophoresis and were not used.

Most of the rabbits developed generalized lymphadenosis during the immunization and some of the glands suppurated. Gastric juice and other antigens have not elicited such strong reaction in this laboratory

Absorption of the anti-saliva serum was performed by adding plasma, amylase fractions or γ_{SS} globulin[1]) to the serum with subsequent immunoelectrophoretic control of the absorbed anti-serum with the corresponding antigen.

2. Other antisera. Horse anti-human serum was obtained from the Pasteur Institute, Paris. Rabbit anti-human serum, anti-γ_{1A} globulin and anti-albumin serum were purchased from the Behringwerke, Marburg Rabbit anti-γ_{SS} globulin serum was kindly provided by N E. Saris, Ph.D Aurora Hospital, Helsingfors. The γ_{SS} globulin had been purified by Cohn fractionation followed by DEAE-cellulose chromatography Rabbit anti-human gastric juice serum was prepared as described earlier (28)

Other material. Human albumin prepared by Cohn fractionation was obtained from the State Serum Institute, Helsingfors. It was further purified by Pevikon electrophoresis (*vide supra*) Human γ_{SS} globulin purchased from Kabi AB, Sweden, was further purified by DEAE-cellulose chromatography

Immunoelectrophoresis. The microtechnique of Scheidegger (27) and the Ca^{++}-containing buffer of Hirschfeld (17) were used. The precipitation lines were visualized by staining with amido black.

When the vitamin B_{12} binding components were to be visualized, ^{60}Co- or ^{57}Co-labelled vitamin B_{12} (obtained from th Radiochemical Centre, Great Britain and N.V Philips-Duphar Netherlands, respectively) was added to the saliva before ultrafiltration. Autoradiography was performed as described earlier (28).

Results

The general immunoelectrophoretic pattern observed with whole saliva using

[1]) γ_{SS} globulin = 7S γ globulin. The former more adequate term has been proposed by Waldenström (33)

anti-saliva serum is shown in Fig 1 The occurrence of the eleven different precipitation lines, numbered from the anodal side, in fifteen individual saliva samples is also recorded. The pattern is dependent on the concentration of the saliva. If the saliva was concentrated more than twentyfold, two additional lines in the β_2 and α regions, respectively were sometimes found Unconcentrated saliva gave only a few lines.

The pattern of a parotid saliva pool obtained by mixing several individual samples is shown in Fig 2. The lines 1

Subject No.	11	10	9	8	7	6	5	4	3	2	1
1	–	+		–	+	–	+	+		+	+
2	+	+	+	+	+		+	+		+	+
3	–	+		+	+	+	+	+		+	+
4	+	+		–		–	+	+	+	+	+
5	+	+	+		+	–		+	+	+	+
6	+	+	+	+		+	+	+	+	+	+
7	+		+	–		–	+		+	+	+
8	+	+	+	+	+		+	+	+	+	+
9			+	+		+	+	+	+	+	+
10	+	+	+	+	+		+	+	+	+	+
11	+	+	+	+	+	–	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	
13	+	+	+	+	+	–	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	
15	+	+	+	+	+	–	+	+	+	+	+

Fig 1 Immunoelectrophoretic pattern f whole saliva, obtained with anti-saliva serum and stained with amido black. The table shows th occurrenc f the eleven precipitation lines in 15 individual saliva samples.

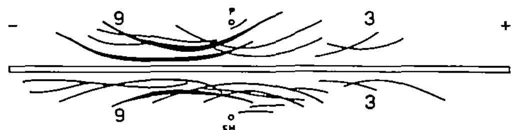


Fig 2 Immunoelect phoretic pattern f parotid (P) and submaxillary (SM) saliva, obtained with anti-saliva serum and stained with amid black Th lines indicated by the number 3 and 9 disappeared fter bsorption f th anti saliva serum with human plasma.

4, 6 and 11, present in whole saliva, seemed to be lacking in parotid saliva. The results obtained with pooled submaxillary saliva are also recorded in Fig. 2. The lines 1, 4 and 8 of whole saliva appeared to have no counterparts in the submaxillary pattern. Thus lines 1 and 4 in whole saliva seem to be derived neither from the parotid nor from the submaxillary glands. These two components might represent either bacterial antigens or products of the other buccal glands or of the mucosa.

In order to find out how many and which of the precipitation lines in the patterns of whole, parotid and submaxillary saliva were due to plasma proteins, the anti-saliva serum was absorbed with human plasma. Only two lines disappeared from all the three secretions, one in the α_1 region and the other in the β region, namely lines 3 and 9 (cf. Figs. 1 and 2), although the immunoelectrophoretic patterns of human plasma developed with unabsorbed anti-saliva serum showed six lines, as seen in Fig. 3. The anti-saliva serum apparently detected only two (lines 3 and 9) of the antigens common to both saliva and plasma in the twentyfold concentrated saliva samples.

Curiously enough, our anti-saliva serum contained no anti-albumin and gave no line when allowed to diffuse against different concentrations of human albumin. However, all our saliva samples contained albumin, demonstrable with anti-albumin serum.

The strongly staining line 9, which disappeared from the salivary patterns after absorption of the anti-saliva serum with plasma, was found to be an immunoglobulin. Both anti-γ_{1A} and anti-γ_{SS} sera developed precipitation lines corresponding to line 9 although hundredfold concentrated saliva samples gave an additional cathodal spur of the line with anti-γ_{SS} serum[*]. Absorption of the anti-γ_{1A} serum with γ_{SS} globulin only reduced the intensity of the precipitation line. The immunoelectrophoretic patterns of saliva obtained with horse and rabbit antihuman sera differ from each other in the $\beta-\gamma$ region as earlier shown by Gabl and Wachter (9). Rabbit anti-human

*) The γ_{SS} globulin concentration in some samples of whole saliva was kindly determined by Dr. Kimmo Aho at the State Serum Institute with the Coombs inhibition technique and was found to be as low as 10-20 μg of γ_{SS} globulin per ml unconcentrated saliva (i.e. about 1/1000 of the concentration in serum).

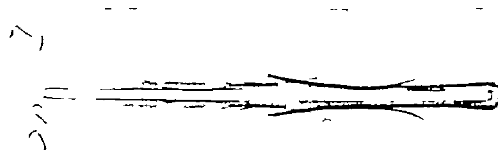


Fig. 3. Immunoelectrophoretic pattern of human serum, obtained with anti-saliva serum. Amid black staining.

serum sometimes showed a γ line in addition to the precipitation line in the β_2 region corresponding to line 9 which was the only line developed in this region with horse anti-human serum. Addition of buffered cysteine or mercaptoethanol to the saliva samples prior to immunoelectrophoresis resulted in no change in the patterns.

In order to identify some of the other proteins in the salivary pattern, the dissolved mucin clot was subjected to immunoelectrophoretic analysis. The result obtained with anti-saliva serum is shown in Fig 4 No lines were produced with anti human serum.

The purified amylase preparations all gave one precipitation line with anti-saliva serum corresponding to line 10. Absorption of the anti-saliva serum with these three amylases caused the disappearance of line 10 only

Although the three amylase preparations emerge as discrete fractions in calcium phosphate chromatography their immunoelectrophoretic behaviour seems to be the same. An amylase line was found in both parotid, submaxillary and whole saliva, staining most strongly in the first secretion

Fig 5 shows an autoradiogram obtained after addition of ^{57}Co-B_{12} to whole saliva in excess of the vitamin B_{12} binding capacity as determined by dialysis. ^{57}Co-B_{12} of high specific activity (100 $\mu c/\mu g$) was also used in a concentration of 3 ng B_{12} per ml saliva. Ten individual whole saliva samples were studied, all behaving similarly and showing only one B_{12} binder with anodic mobility Both anti-saliva and anti-gastric juice sera gave the same precipitation line, the one produced by anti-saliva being broader Anti-human serum gave no precipitation line. The radioactive line did not coincide with any of the lines visualized by protein staining

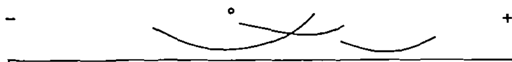


Fig 4 Immunoelectrophoretic pattern of th dissolved mucin clot, obtained with anti-saliva serum. Amid black staining



F g 5. Autoradiographic patterns f whole saliva. ^{57}Co-l belled vitamin B_{12} wa added to the saliva prior t the immunoelectrophoresis. Both anti-saliva serum (AS) and anti-gastric j ic serum (AGJ) wer used.

Discussion

The rabbit anti-human saliva serum pool used in the present study was found to be well suited for the characterization of the human salivary proteins. The separation of the different components was good and little variation was seen in the immunoelectrophoretic patterns of the individual samples of saliva. The poorer resolution obtained by previous investigators who used anti-saliva sera might have been due to the methods they employed to produce their antisera.

The immunoelectrophoretic patterns of saliva obtained with anti-saliva serum do not necessarily reflect the quantitative nor even the qualitative distribution of the different salivary proteins. Although, for instance albumin was shown to be regularly present in saliva, our anti-saliva serum pool contained no anti-albumin. However the number and distribution of the precipitation lines of whole, parotid and submaxillary saliva in the patterns obtained in our study compare fairly well with the results of conventional electrophoretic studies. In free electrophoresis, Patton and Pigman (26) found about ten to twelve components in parotid and submaxillary secretions. Weinstein *et al.* (34) separated parotid saliva into eleven components with starch gel electrophoresis. Fischer *et al* (6) resolved whole saliva into thirteen fractions by paper electrophoresis after removal of the ions by electrodialysis. The β region usually contained the major components of saliva.

The presence of plasma proteins in saliva is a well established fact (5 7 8). The varying number of plasma proteins demonstrated can probably be explained by the different antisera used and also by variations in the protein content of the saliva samples analysed. Our results indicate the presence of at least seven plasma proteins, in agreement with the findings of Gabl and Wachter (9) and Leach *et al.* (22)

The immunoglobulins found in saliva present a problem of their own Both γ_{1M} γ_{1A} and γ_{2S} globulins were reported to be present (9) but our results indicate that the main immunoglobulin found in saliva reacts as a γ_{1A} globulin (line 9) According to a short abstract (31) Tomasi and Ziegelbaum have arrived at the same conclusion. γ_{1A} globulin has also been shown to be the main immunoglobulin in milk and colostrum (15) The explanation of the high ratio of γ_{1A} to γ_{2S} globulin in these three secretions is not known. γ_{1A} paraproteins display a tendency to associate with other plasma proteins, notably albumin (1 16) but possibly also with γ_{2S} globulin The complexes dissociate after the addition of thiols. Such substances had no effect on our salivary immunoelectrophoretic patterns. The well-known fact that all the immunoglobulins contain common antigenic determinants and the fact that the very low concentration of γ_{2S} globulin in saliva might explain the behaviour of our anti-γ_{2S} serum. This antiserum always gave a precipitation line corresponding to the γ_{1A} line with saliva although in more concentrated saliva samples a cathodal spur of this line was sometimes seen. When it was allowed to react with serum, a well-defined γ_{2S} line was the only line seen.

Beerstecher and Altgelt were the first to

demonstrate that saliva binds vitamin B_{12} (2) Gräsbeck (12) subjected human saliva to starch electrophoresis and observed that a single anodal component was responsible for the vitamin B_{12} binding capacity The salivary B_{12} binder was visualized in our autoradiographies by both anti-saliva and anti-gastric juice sera but not by anti-human serum. This same B_{12} binder has also been identified in blood serum by both analytical and preparative methods but in a concentration much lower than in saliva and gastric juice (29) This B_{12} binder corresponds to the non-intrinsic factor-active binder "R" isolated from human gastric juice (13) This B_{12} binder in serum apparently did not give rise to antibodies in rabbits or horses immunized with serum. This B_{12} binding protein has also been found in other body fluids besides those already mentioned (29)

As exemplified by the behaviour of the B_{12} binding protein, the classification of the components of the different extravascular body fluids as plasma proteins or components "specific" to the body fluid in question is arbitrary (14) The sites of production and the turnover patterns of the protein should be elucidated, but few clues to these problems are obtained by descriptive and qualitative immunochemical studies of the present kind.

Summary

Rabbits were immunized with pooled whole human saliva. The resulting antisera and various other antisera were used to produce immunoelectrophoretic patterns of whole, parotid and submaxillary saliva samples. Eleven precipitation lines were obtained with antisaliva serum against whole saliva. Two of these lines were found to be of neither parotid nor submaxillary origin. Precipitation lines corresponding to all the other nine lines detected in whole saliva were found in the patterns of either parotid or submaxillary saliva or of both. Whole saliva contained at least seven "plasma proteins" The most abundant immunoglobulin in saliva behaved as γ_{1A} globulin. Amylase was identified in all the three salivary secretions studied. The components of the mucin clot were also investigated. The salivary vitamin B_{12} binding protein was visualized by autoradiography after addition of radioactive vitamin B_{12} prior to fractionation.

Acknowledgements

Various aspects of this study were supported by grants from the Finska Läkaresällskapet, the Sigrid Jusélius Foundation and the Finnish Research Council for Medical Science and Grant AI-01693 of the U.S. Public Health Service, N.I.H. We wish to thank Miss Tuula Kumpula for capable technical assistance.

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The Effect of Experimental Venous Thrombosis on the Mast Cells and Fibrocytes in the Vascular Wall of the Rabbit

By

Tor Pettersson and Göran Hjelmman

It is generally accepted that the mast cells contain heparin or a precursor of heparin (4 5) and histamine (6 8) Heparin is a powerful anticoagulant and the main effects of histamine are vasodilatation and increased capillary permeability It is understandable that the mast cells have attracted great interest in connexion with thrombotic conditions. The function and significance of these cells are not yet fully understood, however and several different hypotheses have been advanced.

It has been suggested that by releasing heparin the mast cells prevent the growth of a thrombus, thus constituting a defence mechanism against the disease. Observations supporting this view have been made. An increase in the number of mast cells has been reported in the adventitia of dilated haemorrhoidal veins in man (2) and in the adventitia of the uricular marginal vein of the rabbit in experimental thrombosis (3)

Latterly different views concerning mast cell function have been advanced. Riley (9) has proposed that the role of these free connective tissue cells mainly consists in supplying their granules — believed to contain heparin and heparin-bound histamine — to the ground substance of the connective tissue, so contributing to the maintenance of the integrity of the latter Riley suggested that when the connective tissue is exposed to stimulation the mast cells respond by releasing histamine, which stimulates the fibrocytes to phagocytose the granules shed by the mast cells, and that the fibrocytes subsequently deliver the material ingested, perhaps n converted form, to the ground substance (9 1)

This uncertainty with regard to mast cell function prompted us to make further studies on the cell reaction in the vascular wall in experimental thrombosis.

Material and Method

Our material consisted of 23 full-grown rabbits, 14 females and 9 males. Their weight varied between 1850 and 3700 g. Four animals were given an injection of Varicol[R] (Orion, 10 per cent aqueous solution of some fatty acids derived from cod liver oil) in the marginal vein of the left ear. In the remainder 0.5–1.0 ml of Varicocid[R] (Gehe) was injected into the same vein in order to produce exout thrombosis. Seven days after injection the animals were killed under ether anaesthesia and specimens of the thrombosed vein were collected. From the corresponding site in the right ear another specimen was taken as control. After 48 hours fixation in freshly prepared 4 per cent basic lead acetat solution, ordinary embedding in paraffin and sectioning t 10 μ, the preparations were stained in 1 per cent aqueous solution of toluidine blue for 10–15 minutes and mounted in Canada balsam.

Observations and Discussion

In the 4 rabbits given Varicol, the injection resulted in severe necrosis of a large portion of the ear so that the preparations could not be used. In 17 of the 19 animals given Varicocid the injection had the intended effect a clearly discernible thrombosis developing in the marginal vein of the left ear As a rule the thrombosed vein was surrounded by a relatively extensive area of inflammation. One rabbit died 6 days after the injection, and in another no thrombosis was observable.

In all preparations from the marginal vein of the control ear only a few mast cells were seen in the tunica adventitia and the surrounding connective tissue. As a rule they were richly granulated. Extracellular granules were not detectable. In the fibrocytes no metachromatic granules were observed (Fig 1)

In the preparations from the walls of the thrombosed vessels, cells with metachromatic granulation were mostly seen in large numbers in the tunica adventitia. Some of these were typical mast cells. They contained numerous granules and were of the same size as the mast cells in the control preparations. Occasionally extracellular metachromatic granules were observed in the vicinity of the mast cells. Furthermore, a large number of cells of almost the same size and many smaller cells containing metachromatic material were present. Many of these cells had the same appearance as the fibrocytes. The amount of metachromatic granulation in them varied considerably Mast cells and other connective tissue cells containing metachromatic material were observed in abundance not only in the vascular wall, but also far outside in the surrounding connective tissue. In addition fibrocytes without any metachromatic granules were seen in large numbers (Fig 2)

In this study we did not determine the number of mast cells per unit volume, but our observations undoubtedly corroborate the view previously put forward by Hjelmman and Hjelmman and Wegelius (2 3) that the mast cells in the vascular wall increase in number in connexion with thrombosis. It is obvious, however that only a portion of the cells containing metachromatic material are true mast cells. Many such cells, in particular the smaller ones, are obviously fibrocytes, or possibly some other type of connective tissue cell. If they are packed with granules they often bear a

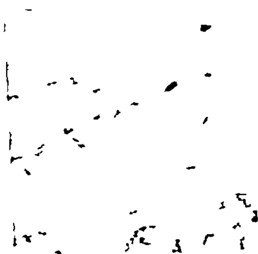
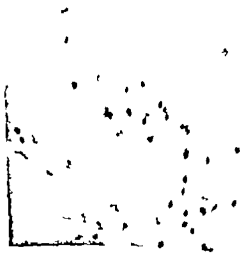


Fig 1 Control ear T the left part f th vascular lumen. Above to th right, two mast cell rich in granules. x 100



F g ... I jected ea The vei is a n at th middle of th picture A larg number of cells containing metach omatic g nul ... the vascular wall and i the surrounding nective tissue x 100



Fig 3 Mast cell and other connective tissue cell ontaining metachromatic granule are seen nea thrombosed vein 400



F g 4 A definit ... smaller cell containing les Oil immersion ...

striking resemblance to mast cells, and it is very difficult to distinguish them with certainty from the latter as appears from Figs. 3 and 4 It is not easy to decide whether these cells have taken up granules released by the mast cells or have formed their metachromatic contents themselves. We observed many mast cells with extracellular granules nearby Smith and Lewis (10) and Riley (7) have previously shown that if loose connective tissue is exposed to stimulation, the mast cells react by releasing their granular contents into the surrounding connective tissue. In the present study the majority of the fibrocytes were filled with granules, like mast cells the granular substance being situated in the cytoplasm.

Our observations concerning the occurrence of metachromatic granules in the fibrocytes in the wall of thrombosed vessels and in the surrounding connective tissue are in good agreement with the result obtained in other investigations in which the connective tissue was exposed to some form of stimulation. Higginbotham (1) showed that a slight trauma to the skin of mice leads to degranulation of the mast cells and to the appearance of metachromatic granules in the fibrocytes. When S^{35}-labelled granules isolated from mast cells were injected they could also be traced in the fibrocytes

Summary

In experimental venous thrombosis in the rabbit the mast cells in the tunica adventitia of the vascular wall and the surrounding connective tissue increased in number Simultaneously metachromatic granules were observable in a large number of other connective tissue cells, particularly in cells which were identified as fibrocytes. It is often difficult to distinguish between mast cells and fibrocytes containing numerous granules.

Acknowledgement

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Tularaemia in Finland

By

Gösta Björkenheim Louis Bardy Erik Lindholm and Aimo Salminen

Pasteurella tularensis is pathogenic for a number of animals, *e.g.* hares and rabbits and other rodents, insectivora, sheep and some birds, which are the reservoirs of the bacterium in nature. The human being may be infected with *P. tularensis* through direct contact with infected animals or with their excreta, but often the bacterium is transmitted by blood-sucking arthropods, *e.g.* ecto-parasites of infected animals, biting flies (*Chrysops*) and ticks (*Dermacentor Ixodes*) the latter probably also forming a natural reservoir of *P. tularensis* (17)

In man the disease may run its course in one of three different forms:

I. A local, ulceroglandular form with ulceration, lymphadenitis and fever The formerly used terms ulcerous, glandular oculo- stomato- and bdominoglandular tularaemia refer to this form of the disease, which has a fa ourable prognosis and in which deaths are rare. The course may however be very protracted.

II A diffuse, febrile form where the disease may be complicated by pneumonia, pleurisy rash, herpes, erythe ma nodosum, encephalomeningitis, hepat itis, arthritis or thrombophlebitis, while ulceration and lymphadenitis are lacking The prognosis in this group is not so favourable. Mortality up to 10 per cent was reported before the antibiotic era. Pulmonary tularaemia is particularly dangerous, having a mortality up to 60 per cent if not treated by specific means.

III An abortive form in which the disease runs its course without symptoms or is characterized by slight fever headache and fatigue (5 10)

Streptomycin is the drug of choice in the treatment of tularaemia. Tetracyc lines and chloramphenicol have a weaker effect and relapses are common Penicillin and sulphonamides are ineffective.

The diagnosis can be confirmed by agglutination tests or skin testing Agglu tinating antibodies against bacterial sus-

persons of *P. tularensis* appear in the blood during the second week of the disease. Peak titres are reached during the fifth or sixth week. The antibodies begin to disappear after some months. An intradermal reaction against dead microbes can be elicited within a few days of the onset of the illness and the response remains positive for years. It has not been possible to cultivate *P. tularensis* directly from human specimens, but inoculation of guinea pigs with infected material may often be successful. Infection among laboratory staff is not uncommon.

P. tularensis was discovered by McCoy (12) in 1911 as the cause of an epidemic disease among rodents in the Tulare district of California. Vail (24) described the first case of human tularaemia in 1914. Francis (4) showed that tularaemia was identical with a disease earlier known in the United States to affect hunters, poulterers, shearers, *etc.* The disease has subsequently been found in several countries. It has been quite common in North America, Japan and Siberia. In the nineteen-twenties tularaemia was observed in Germany and France and in the Balkans. It has also appeared in the Leningrad area and was not so very rare on the eastern front during the Second World War.

As regards Scandinavia, it may be mentioned that the first cases of tularaemia were reported from Norway by Bryn (2) and Thjotta (18, 19) in 1930 and one year later the disease was recognized in Sweden by Granström (6). In Norway Thjotta (20, 22) had observed almost 100 cases up to 1941. Since then the problem of tularaemia has not been more closely examined in Norway. From the epidemiological point of view it was of interest that in Norway Thjotta (21) found cases in which the infection had in all probability been contracted from the lemming. He also supposed that one form of the "lemming diseases" which occur among the human population during the years of lemming migrations, might be caused by *P. tularensis*. This hypothesis was confirmed by Olin (13, 14) in Sweden who isolated five strains of *P. tularensis* from lemmings and one strain from their ectoparasite, *Megabothris rectangulatus* during an outbreak of human tularaemia in Swedish Lapland. Olin also succeeded in isolating *P. tularensis* from the mosquito *Aedes cinereus*. On the basis of epidemiological features he considered mosquitoes to be the most important vector of tularaemia in Sweden.

In Sweden, tularaemia has been the subject of many investigations. Outbreaks of human tularaemia have been described by Granström (6), Carlberg (3), Olin (13, 14), Tveterås (23), Ljung (11) and Björkman *et al.* (1). In total about 1100 cases have been found most of them from Central Sweden. On the other hand, tularaemia has not been reported from Denmark.

In contrast with Sweden and Norway the literature concerning tularaemia in Finland is scanty. In 1932, Sievers (16) found in a serological survey for tularaemia agglutinins a clearly raised titre in a patient with fever of unknown origin. Moreover, he found slightly raised titres in some subjects and an unspecifically increased agglutination in some cases of undulant fever.

In 1939 B. Grönroos (7) reported a case in which the diagnosis ulceroglandular

tularaemia was quite certain, the agglutination titre for tularaemia was 1 1280 The patient had caught the infection through a tick bite near Helsinki on an island where many dead hedgehogs had been found

Thus only two clinical cases of tularaemia have hitherto been reported in Finland. After 1938, there were no reports of the occurrence of tularaemia until the next cases were established in 1959

Case Records

In 1959 in the Kymi valley near Kotka, two cases of tularaemia were observed In 1961 we found third patient living 20 kilometres away from the first two. A description of these cases is given below

Case 1 Surgeon, male, 43 (Fig.1) H fell ill in the beginning of August, 1959 with fever 39 40 C which lasted for ten days, during which he observed small necrotic wound on his right ankle and swollen and sore glands proximally on his right thigh He took tetracycline for week The temperature decreased somewhat, but he was still tired and the glands were tender Soon the fever rose again for week and at the same time numerous new and very tender nodes appeared box the right groin and in the right iliac fossa. His general condition was strongly affected. H had severe aches in his right ankle several times. The diagnosis was quite unknown t this stage. W considered the possibility of *Toxoplasma* hospital infection, because the patient, some months before, had removed gland from patient with histologically and serologically verified toxoplasmosis, but no serological evidence of toxoplasmosis was obtained. Consultation of the "list of existing infectious diseases suggested the possibility of tularaemia, since it seemed to be clinically the most likely diagnosis. Serological tests now showed positive agglutination titre for tularaemia, maximum 1:320. A glandular biopsy from the groin showed changes typical of tularaemia (Professor H. Teir M.D)

The patient was now treated with doses of 0.5 g dihydrostreptomycin and 0.5 g streptomycin sulphate daily for month. His temperature gradually returned to normal. The swelling of the lymph nodes slowly subsided and there was temporary suppuration. A guinea-pig inoculation test from the pus was negative. A slight glandular

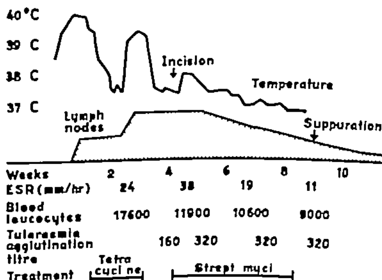


Fig 1 — Clinical course of the disease in case 1

swelling remained for year The patient did not overcome his fatigue until the following summer

When we started to suspect tularaemia the patient remembered that short time before he fell ill he had found his dog playing with dead hare. H had taken the carcass from the dog and thrown it away At the same time he had been stung by gadfly on his right ankle.

Case 2. Labourer male, 37 neighbour of patient 1 He fell ill at the same time as the first patient with bilateral inguinal lymphadenitis. H was treated in another hospital with mixture of streptomycin and penicillin. The gland abscesses were incised. After this he recovered. The case was brought to our attention and blood samples were drawn six weeks after the onset of the illness. At that time the agglutination titre for tularaemia was 1·640 and some weeks later 1 1280.

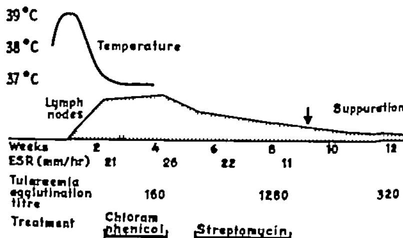
Case 3. Female, 12 (Fig. 2) In the autumn of 1961 she caught wild mouse in haystack. The mouse bit her in the left middle finger and escaped. A week later she developed slight wound necrosis at the site of the bite and fever up to 39°C, which lasted for week. After that she developed lymphangitis and very tender lymph nodes in the elbow region and on the inside of her upper arm. Chloramphenicol affected the glands only slightly but after treatment with streptomycin for three weeks they gradually disappeared. I this case, too, some suppuration occurred. The highest tularaemia agglutination titre was 1 1280.

Table I Results of agglutination tests for tularaemia in our three cases.

Case number	Date of blood specimen	Titre of agglutinating antibodies to *P tularensis*
1	Sept. 4 1959	1:160
	Sept. 11 1959	1:320
	Sept. 25 1959	1:320
	Oct. 7 1959	1:320
	June 8, 1961	1:20
2.	Sept. 14 1959	1:640
	Oct. 7 1959	1 1280
3.	Oct. 19 1961	1 160
	Nov 15, 1961	1 1280
	Dec. 16, 1961	1:320

The tularaemia agglutination titres in these three cases are shown in T ble I. No *Brucella* agglutinins were detectable.

Remarks The delay in making the right diagnosis is often considerable in sporadic cases of rare disease. In fact, it is difficult to recognize disease one has never been acquainted with and especially if the only thing one knows about it is that it does not occur in the country in which one is living. Hence it is understandable that in case 1 it took long time before the correct diagnosis was made. That previous acquaintance with the disease facilitates rapid diagnosis ppears from cases 2 and 3



F g 2 — Clinical ourse f the diseas in ase 3

Other cases in Finland. In 1961 in Turku, J Grönroos (8) found some slightly positive agglutination titres for tularaemia and in one patient titre of 1 160. In that case the clinical picture resembled tularaemia. In 1962, Hernberg (9) observed serologically verified case of tularaemia in Helsinki. The patient was an elderly woman who had been taking care of sick mouse before she contracted lymphadenitis. Both the patient of Hernberg (9) and that of B Gronroos in 1938 (7) had been treated by many physicians before the correct diagnosis was made.

Discussion

From time to time since the 1930' bacteriologists have looked for tularaemia in Finland. They have been astonished at the rarity of the disease in this country in spite of its rather frequent occurrence in Sweden and Russia. Yet the 7 cases of tularaemia hitherto observed in Finland suggest that the disease, though rare, may be of more than sporadic occurrence here. It is possible that in Finland quite a number of undiagnosed cases of tularaemia have occurred, partly of the local form, and perhaps above all of the diffuse and the abortive forms.

This possibility is to some extent supported by Severs (16) and J Grönroos (8) screening tests and also by a recent screening in the district of Kotka. Here Pätiälä, Salminen and Björkenheim (15) found tularaemia agglutinins in 17 out of 109 people tested. The highest titre in that material was 1 80, in two cases the titre was 1 40 in si 1 20 and in eight 1 10

The diagnosis is easily made in the typical case of ulceroglandular tularaemia within an endemic region Outside such a region the disease often remains undiagnosed. But even in tularaemia regions it may be difficult to diagnose the diffuse and abortive forms of the disease. These are obviously more common than the ulceroglandular form; yet the latter is more often notified. On the basis of intradermal tests, Ljung (10) supposes that 10 per cent of the adult population in Swedish endemic districts have been infected with tularaemia.

To what extent *P tularensis* occurs among animals in Finland has not yet been elucidated. We were unable to find the bacterium at the examination of 26 hares shot in the district of Kotka in 1961 and not even in some wood mice, *Apodemus sylvaticus* caught in the same haystack where our patient 3 was bitten.

It remains to explain how *P tularensis* has been spread to Finland. A direct continuous or sporadic invasion by land from the south-east through the intermediation of rodents or of their ticks seems to be the most important, perhaps even the chief invasion route of the microbe. But we also want to call attention to the possibility that *P tularensis* at least sporadically could be brought to Finland by birds, either directly or by their ticks.

Summary

Only two clinical cases of tularaemia have been previously reported in Finland both in the 1930's. An account is given of three cases which were observed in the district of Kotka during the years 19[illegible] 1961 In addition, reference is made to two other recent cases, which were diagnosed in Helsinki and Turku. Thus only 7 clinical cases of tularaemia are known from Finland On the basis of these cases and some serological screenings it

is suggested, however that tularaemia is not so rare in Finland as has hitherto been assumed but the disease has mostly remained unrecognized.

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Diphasic Tick borne Meningo-encephalitis, Kumlinge Disease in the Åland Islands

Diagnosis, Clinical Features, and Epidemiology

By

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Diphasic tick-borne meningo-encephalitis occurs at least in the south-west and the south-east of Finland (7 10) The region of heaviest infection seems to be the Åland Islands in the south west of the country where the disease locally called Kumlinge disease has apparently occurred since at least 1942 (9) Although the distribution of the tick *Ixodes ricinus* (8) which is regarded as the vector of the virus, and the ecology of tick-born encephalitis viruses in this region (1 11) have been studied for several years no detailed description of the clinical features of Kumlinge disease has so far been published. The purpose of this paper is to fill this gap and at the same time to touch upon some problems of the virological diagnosis and epidemiology of the disease.

Material and Methods

During the years 1959 1960 and 1961 altogether 24 patients were admitted to the Medical Department of the Åland Central Hospital with symptoms of aseptic meningitis or meningo-encephalitis and antibodies to tick-borne encephalitis virus. In one further case with similar findings the patient was treated at the Medical Out Patient Department. In addition, two patients with similar symptoms and likewise antibodies to tick-borne encephalitis virus, who had spent their vacation in the Åland Islands, were seen at the University Department of Virology These patients, 27 in all, form the basis for the present study

The patients were subjected to a general medical examination, including blood picture and differentiation count, erythrocyte sedimentation rate chest X-ray lumbar puncture and the cardiolipin reaction for syphilis. In the following text the omission of a reference to the result of some investigation indicates a normal finding For the virological investigations, see below

Table I. Results of haemagglutination inhibition test (HI) and complement fixation test (CF) with tick-borne encephalitis virus in 27 patients with meningo-encephalitis.

Patient No.	Day after onset of first phase of illness	HI	CF
1	16	320	20
	28	640	10
2	10	160	10
	22	320	40
	150	320	10
3	13	640	
	26	640	10
4	7	320	10
	18	640	40
5	11	320	10
	17	320	40
	30	320	40
	11	160	20
6	16	160	<5
	26	320	10
	41	320	20
7	13	80	10
	21	160	20
8	6[1]	<10	<5
	16	160	10
9	15	160	10
	25	320	40
10	25	640	10
	33	640	10
11	19	640	<5
	25	320	5
	38	640	5
12	27	320	20
	48	640	20
13	21	1280	<5
	27	1280	20
	35	640	20
	44	640	20
14	22	320	<5
	29	320	10
	36	640	5
	50	1280	10
	113	160	20
15	21	320	<5
	32	640	10
16	10	80	<5
	19	320	10
	26	160	20
17	14	320	10
	22	320	40
	30	320	80
	50	640	80
18	23	320	10
	37	320	20
	39	320	20
19	21	320	20
	36	640	20
20	20	320	20
	28	320	80
	37	1280	160
21	20	160	20
	26	160	40
	38	640	320
22	17	320	160
	38	160	80
	62	160	40
23	14	320	<5
	22	640	320
	32	160	160
24	11	80	<5
	16	160	<5
	23	160	20
	30	160	40
	38	160	40
	61	80	40
25	22	40	40
	26	80	40
	34	80	40
	48	160	20
26	22	80	10
	29	40	40
	36	80	40
27	6[1]	<10	<5
	[illegible]	[illegible]	[illegible]

Specimens for sero-diagnostic tests

In general, blood specimens were taken on the second day after hospitalization and about 10 days later From several patients third blood sample was taken 1—3 weeks later and in some instances even fourth and fifth specimen, as indicated in Table I. Stool specimens and cerebrospinal fluid were obtained during the acute illness for isolation of possible enteroviruses.

Virus-diagnostic methods

I 1 tI n

Isolation of tick-borne encephalitis irus was done by inoculating serum from acute phase blood intracerebrally into 10—12 g mice.

Attempts to isolate irus from faecal specimens in tissue cultures were made in 1959 with HeLa cells, and from 1960 onwards with U-cells, continuous line of human amniotic cells (13) and technique described elsewhere (12) U-cells are susceptible to the different types of polioviruses and the B-group of Coxsackie iruses and Coxsackie A 9 (13) among others, but practically non-susceptible to many other enteroviruses.

S l gi l t t f ti k b a ph liti i u

The haemagglutination inhibition test was performed according to the method described by Clarke and Casals (2) with some modifications. The haemagglutinin was prepared in tissue culture from the Belyanchikov strain by protamine treatment and thermal inactivation as described earlier (14).

The complement fixation test was performed with tissue culture antigen prepared with the local strain A 52 of tick-borne encephalitis virus in U-cells (17). The tests were made with four units of the antigen by the test tube method (4)

Results

Aetiological diagnosis

The results of the serological tests with diphasic tick-borne encephalitis virus are presented separately for each of the 27 patients in Table I and summarized in Table II. An increase in antibody in one or both of the tests used was demonstrated in the sera of *18* patients only In 6 cases, there was a fourfold *or* greater increase in both the haemagglutination inhibition test and the complement fixation test. From the acute phase blood specimens of 2 of these patients, who initially had no demonstrable antibodies a virus of the tick-borne encephalitis group was isolated. These 6 patients may thus be regarded as virologically verified cases of tick-borne meningo-encephalitis. In 11 patients, there was no significant change in the level of the haemagglutination inhibition titre, but a fourfold or greater increase in the comple-

Table II. Summary of the serological pattern and the virus isolation in 27 patients with meningo-encephalitis and antibodies to tick-borne encephalitis virus.

Haemagglutination inhibition reaction	No. of patients	Complement fixation reaction	No. of patients	Virus isolated, no. of patients
Rise[1]	7	Rise[1]	6	2
		No change	1	
No change	20	Rise[1]	11	
		No change	9	
Total	27		27	2

Only fourfold or greater changes in titre are recorded

ment fixation test. It is known that in the complement fixation test the titre increases in a later stage of the disease than in the haemagglutination inhibition test (6). Thus these 11 patients were also undoubtedly infected with tick-borne encephalitis virus in connexion with the present illness, but they were apparently admitted to the hospital at a later stage of the infection than the afore-mentioned 6 patients. The results in the single patient with a demonstrable increase in the haemagglutination inhibition test only are not in line with this reasoning. As there was a clear increase in the haemagglutination inhibition test, however, this patient has also been included in the group of virologically verified cases of tick-borne meningo-encephalitis.

In addition, it may be mentioned that attempts to isolate enteroviruses from the stools of 11 of these 18 patients were negative. Although no conclusive investigations were made for all enteroviruses the serological tests with tick-borne encephalitis virus justify the diagnosis of tick-borne meningo-encephalitis in at least these 18 patients. These patients will therefore serve as the basis for a discussion of the clinical features of tick-borne meningo-encephalitis as it occurs in the Åland Islands — the Kumlinge disease.

The remaining 9 patients with positive tests but without a significant antibody

Fig. 1. Map of the Åland Islands.
● = *virologically verified case*
○ = *virologically unverified case*

increase have undoubtedly on some occasion been infected with tick-borne encephalitis virus. The time relationship between the infection and the present illness cannot be determined, however from the serological data available. Serological surveys in the region have shown a fairly high incidence of antibodies in the population of some islands (15) and the positive tests may thus be due to a previous infection.

Regarding the possibility of some other aetiology attempts to isolate enteroviruses from 5 of these 9 patients gave negative results. This seems in these 5 cases to exclude at least poliomyelitis and some of the Coxsackie viruses. The methods used, as already mentioned, were not suitable for the isolation of other enteroviruses. No serological tests were performed with this possibility in mind, however and the aetiology of

Table III. Data from the histories of the 27 patients with meningo-encephalitis and antibodies to tick-borne encephalitis virus.

	Patient No.	Age, years	Sex	Month and year of onset of illness	Tick-bite[1]	Probable place of infection
Virologically verified cases	2	11	m	VI –1959	(+)	Föglö
	4	16	f	VII –1959	(+)	Jomala
	5	15	f	VIII–1959	(+)	Eckerö
	6	50	f	VIII–1959	(+)	Vårdö
	8	53	m	VII –1960	+	Mariehamn
	9	48	f	VII –1960	(+)	Kumlinge
	13	43	m	VIII–1960	+	Sund
	14	28	m	VIII–1960	(+)	Hammarland
	15	59	m	VIII–1960	+	Kumlinge
	16	23	m	IX –1960	(+)	Kumlinge
	17	15	m	IX –1960	+	Kumlinge
	20	51	f	IX –1960	+	Lemland
	21	67	m	VII –1961	+	Mariehamn
	23	58	m	VII –1961	–	Kumlinge
	24	45	m	VIII–1961	(+)	Kumlinge
	25	25	m	VIII–1961	(+)	Lemland
	26	44	m	IX –1961	+	Iniö, Äselholm
	27	52	m	VIII–1961	+	Kumlinge
Virologically unverified cases	1	7	m	VI –1959	+	Mariehamn
	3	21	m	VI –1959	+	Finström
	7	8	f	IX –1959	(+)	Kumlinge
	10	68	f	VII –1960	(+)	Föglö
	11	24	m	VII –1960	+	Föglö (poss. Kökar)
	12	55	m	VII –1960	+	Iniö, Äselholm
	18	6	f	IX –1960	+	Lemland
	19	25	f	IX –1960	(+)	Lumparland
	22	11	f	VII –1961	(+)	Hammarland

[1] + = Certain tick-bite a few weeks before phase I.
(+) = Tick-bites many times during the summer in question, but exact time not stated.

these cases therefore remains obscure from a virological standpoint. The clinical data of these patients will therefore be discussed separately

Clinical features

Virologically verified cases of tick borne meningoencephalitis

The pertinent data of these cases are summarized in Tables III IV and V In the 18 virologically confirmed cases every patient remembered having been infested with ticks many times during the summer in question, and 9 had with certainty been attacked "recently" i.e within a few weeks of the onset of the illness.

A typical feature of the disease was the diphanicity of the illness. Only for one of the patients in this group (No 8) could a history of a first phase not be obtained. This patient, incidentally is one of the two from whose blood the virus was isolated

The first phase of the illness was characterized by vague symptoms of "cold or flu" dizziness, slight headache, and pain in the limbs. A slight rise in body temperature was common Generally the patients were not in bed during this phase but stayed away from work for 2 or 3 days. This phase lasted from 1 to 6 days, on average about 4 days. Only one patient sought medical aid during the first phase, but at that time the nature of the disease was unrecognized and no specimens were taken until the second phase, when th patient again applied for medical treatment.

The interval lasted an average of about 9 days, the range being 3—15 days. During this period, the patients resumed their usual activities, but none felt entirely well

The second phase was generally the one which led the patients to consult a physician. The main complaints were of headache and fever There was lassitude and fatigue. The fever was around 39 C and its duration averaged 6 days (see Table IV)

At the general examination the patients displayed a picture of acute febrile infection. Fifteen had neck rigidity of a kind which was a fairly typical sign of the illness. The neck submitted to flexion actively as well as passively but only to about 30 degrees, after which active flexion was impossible. When passive flexion was attempted a "spring-like" or elastic resistance was felt, which prevented full flexion and caused the patient moderate pain. Only one patient (No 15) displayed marked rigidity of the neck with some tendency to opisthotonus and complete inability for passive or active flexion This patient was the only one who had a paresis (see below) Of the hospitalized patients in this group, there was only one without any neck rigidity at all (No 21) The rigidity as a rule, disappeared with the fever Other symptoms from the central nervous system were sparse. Photophobia was recorded in 2 patients, vomiting in 4 patients, somnolence in 1 One patient (No 15) had a transient left facial paresis. This paresis appeared at the height of the second phase of the illness, and disappeared completely within 2 weeks. One patient had conjunctival irritation. All these symptoms

Table IV Clinical data of the 27 patients with meningo-encephalitis and antibodies to tick-borne encephalitis virus.

	Patient No.	Age years	Phase I, duration, days	Interval, duration, days	Stay in hospital, days	Maximal body temperature C	Duration of fever, day	Maximal blood leucocyt count/mm³	Maximal E.S.R. mm/hour	Rigidity of neck, see text	Remarks
Virologically verified cases	2	11	3–4	3–4	13	40.3	1	13,600	26	+	
	4	16	3	3–4	18	39.4	6	11,300	20	+	Photophobia
	5	15	5	5	17	37.8	2	6,200	21	+	
	6	50	3–4	9	26	38.9	4	9,200	31	+	Photophobia, vomiting
	8	53	—	—	14	39.0	8	4 100	[illegible]	+	"Monophasic" Virus isolated from blood
	9	48	4	4	15	39.0	8	8,400	56	+	
	13	43	~2	14	31	38.4	5	12,400	21	+	Pneumonia
	14	28	4	14	18	39.0	6	10,300	13	+	Redness of face marked somnolence vomiting, very ill
	15	39	3	10	22	40.2	6	8,200	31	(+)	Transient unilateral facial paresis, vomiting, *marked* neck rigidity very ill
	16	23	4	7	17	39.9	6	12,500	19	+	Redness of face
	17	13	1–2	8	20	39.7	5	8,000	20	+	Redness of face
	20	31	5	7	23	39.0	10	4,800	10	+	
	21	67	~3	14	23	39.8	6	8,000	50	−	
	23	58	4	9	28	39.8	7	12,600	35	+	
	24	45	2	7	32	40.5	6	7 700	12	+	Very ill
	25	25	6	15	32	40.0	6	6,600	38	+	
	26	44	3	14	18	40.3	6	13,200	10	+	Vomiting
	27	32	5	8	—	37.8	4				Not hospitalized. Virus isolated from blood
Mean		39	4	9	22	39.3	6	9,100	28		
Range		11–67	1–6	3–15	13–32	37.8–40.5	2–10	4 100-15000	12–56		
Virologically unverified cases	1	7	3	9	15	39.3	5	12,400	42	+	Vomiting
	3	21	7	3	15	38.8	5	7,000	25	+	
	7	8	8	3	17	40.6	6	13,000	33	−	Vomiting
	10	68	?	7	31	37.8	—	12 700	38	(+)	Cerebral haemorrhage during phase II Vomiting (before haemorrhage)
	11	24	1	13	22	39.1	2	7,200	23	+	Vomiting
	12	55	6	4	4	39.6	3	10,000	40		Treated elsewhere
	18	76	7	5	22	38.0	5	6,500	32	?	Wife had tick-borne encephalitis 1959 Admitted late in phase II
	19	25	2	8	1)	38.5	10	7,600	12	+	
	22	11	2	9	24	39.4	8	6,700	50	+	
Mean		33	4	7	19	39.0	5	9,200	37		
Range		7–68	1–8	3–13	14–31	37.8–40.6	3–10	6,700-13000	12–52		

[1] In bed at home for 14 days.

and signs, with the exception of the paresis, disappeared within the first week of the second phase.

The disease was in some cases accompanied by a slight leucocytosis in the blood (Table IV). The erythrocyte sedimentation rate was moderately elevated (Table IV) with a peak one week after the beginning of the second phase. It returned to a normal level within 2 weeks of this peak.

The findings in the cerebrospinal fluid are recorded in Table V. All 16 patients whose cerebrospinal fluid was examined

Table V. Cerebrospinal fluid findings from the 27 patients with meningo-encephalitis and antibodies to tick-borne encephalitis virus.

	Patient No.	Cells per mm³		Pressure, mm of fluid	Nonne	Pandy
		Mononuclear	Polymorphonuclear			
Virologically verified cases	2	54	19	Not rec.	±	+
	4	67	38	240	+	++
	5	29	24	165	±	+
	6	35	56	250	±	+
	8	8	6	125	±	+
	9	175	57	210	+	++
	13	Refused lumbar puncture				
	14	61	28	120	+	++
	15	66	43	160	+	++
	16	41	105	170	+	+++
	17	19	60	280	±	+
	20	41	65	Not rec.	±	+
	21	11	25	190	+	++
	23	33	85	80	±	+
	24	44	31	150	±	+
	25	93	33	140	+	++
	26	21	61	310	+	++
	27	Subclinical infection				
Mean		50	49	185		
Range		11–175	6–105	80–310		
Serologically verified cases	1	51	60	Not rec.	+	++
	3	43	33	Not rec.	+	++
	7	45	120	200	+	++
	10[1]	6	4	180	+	++
	11	85	63	200	+	++
	12	24	4	Not done		
	18	107	99	200	+	++
	19	CSF not examined (Outpatient)				
	22	16	25	Not rec.	±	±
Mean		46	52	195		
Range		6–107	4–120	180–200		

[1] 7 days later this patient had a cerebral haemorrhage and the cerebrospinal fluid became haemorrhagic. Later on xanthochromia.

had an elevated cell count in the beginning of the second phase. The cell count fell to normal 1—4 weeks later Both mononuclear" and polynuclear" cells were seen the type of the dominant cell was not constant. The pressure of the cerebrospinal fluid was in most cases moderately elevated The sugar content was normal Quantitative protein measurements were not performed owing to lack of facilities. The Nonne and Pandy reactions, however were positive.

The clinical symptoms and signs disappeared within 13—32 days, the stay in hospital averaged 22 days. Late symptoms were usually absent, but one patient complained of severe fatigue for about 2 months after discharge and another had lower back pain which persisted for about half a year

To summarize the general picture of the virologically confirmed cases of tick borne meningo-encephalitis was as follows: a person who has suffered tick-bite during the summer in question has a mild gener al infection which lasts for 2—6 days. A week or two later he runs a high fever and has a moderate to severe headache. An "elastic neck rigidity is often noted. The cerebrospinal fluid has an elevated cell count and its pressure is usually higher than normal. The fever subsides after about one week, and the symptoms and signs disappear within 2—3 weeks. Late symptoms are usually absent.

V rologically unver f ed cases

The pertinent data of these patients are also summarized in Tables III IV and V As far as the main clinical features are concerned, an obvious similarity to the patients of the virologically verified group is noted.

All the patients in the virologically unverified group had been infested w th ticks many times during the summer in question and 5 were with certainty attack ed "recently i.e within a few weeks of the onset of the illness. The age distri bution of the patients in this group corresponds to the distribution in the previous group.

The first phase was characterized by the same symptoms as in the previous group and its duration was similar

The interval was somewhat shorter on the average than in the first group. The patients resumed work as in the first group although they did not feel com pletely fit.

The second phase had the same charac teristics as in the first group. The same is true regarding laboratory tests and cerebrospinal fluid findings. The typical neck rigidity was present in 5 of these patients.

One of the patients in the virologic ally un verified group (No. 10) had a cerebral (subarachnoid) haemorrhage in the middle of the second phase of the illness. The cerebrospinal fluid became haemorrhagic, there was marked rigidity of the neck and convulsions occurred No pareses were seen and the patient recovered w thin 3 weeks. It is difficult to know whether this condition was caused by virus infection or not. The fact that the patient had a history of minor cerebral accidents might be taken to suggest that the entire illness was caused by cerebrovascula rteriosclerosis. This seems unlikely however in view of the clear d phasicity of the illness and the clinical

findings prior to the haemorrhage (Tables III, IV and V)

It is obvious that the clinical picture of these virologically unverified cases, with the one exception just mentioned, is very similar to that of the virologically verified cases.

Treatment

The patients were treated with bed rest and mild analgesics and sedatives. In many instances, therapeutic lumbar puncture was performed to alleviate the symptoms of increased cerebrospinal fluid pressure

Epidemiology

The archipelago of the Åland Islands is situated around latitude 60°N in the Baltic Sea between the mainlands of Sweden and Finland. It consists of the main island of Åland, about 6 000 large islands and innumerable smaller islands, rocks and skerries. The resident population is about 22,000 The first cases of tick borne meningo-encephalitis in the area were reported from the islands of Kumlinge, hence the local name Kumlinge disease (9) The distribution of the 18 virologically confirmed cases of tick borne meningo-encephalitis and the 9 virologically unverified cases is illustrated in Fig 1 The cases are scattered all over the archipelago which is in good accord with the known distribution of the virus. Eleven of Åland's 16 parishes are represented as sites of infection.

The seasonal distribution of the cases is seen in Table III The cases are distributed between June and September Although no patient of the virologically unverified group was taken ill in August, no significant difference in the seasonal distribution of the cases can be observed between the two groups. No familial cases occurred in the present material.

Discussion

The 27 patients in this study presented a clinical picture which would justify the clinical diagnosis of Kumlinge disease. Virologically however they can be divided into 2 groups. the patients of the first group showed a definite rise in tick borne encephalitis antibodies either in the haemagglutination inhibition test or in the complement fixation test or in both during the present illness; the patients of the second group showed no significant change in antibody titre.

A fourfold or greater increase in haemagglutination inhibiting antibodies seemed to be rare, whereas such an increase in the complement fixation test was demonstrated in 17 out of the 27 patients. Thus haemagglutination inhibiting antibodies apparently appear earlier than complement fixing antibodies, which is in line with the findings of Kunz and Moritsch (6) The haemagglutination inhibition test is therefore suitable for screening purposes, but it does not usually give information about the time relationship between the infection and the illness proper The complement fixation test, however seems to provide an answer to this question and therefore seems to be the method of choice for the diagnosis of acute cases of tick-borne meningo-encephalitis. The diagnosis is further confirmed by a positive virus isolation. This can rarely be done, however as the patients are usually seen in a phase of

the illness when the viraemia has disappeared and antibodies to the virus are already present.

The first group, comprising 18 of the 27 patients, thus comprises verified cases of tick-borne meningo-encephalitis, whereas the aetiology of the illness in the second group of 9 patients, *i.e.* those showing no change in antibody and from whom no virus was isolated, remains obscure. The fact that these 9 patients all had antibodies to tick-borne encephalitis virus, whereas the mean frequency of such antibodies is only 15 per cent among the population of Åland (15) that they had all been infested with ticks during the summer in question and that the clinical and epidemiological pictures accord well with a diagnosis of tick borne meningo-encephalitis seems, however to suggest that at least some of them also represent cases of Kumlinge disease. The question of the aetiology of these cases, which would merit a thorough study will, however be disregarded here for the sake of simplicity and the evaluation of the features of Kumlinge disease is thus based on the verified cases alone.

The course of the diphasic tick-borne meningo-encephalitis as it occurs in the Åland Islands — the Kumlinge disease — is comparatively mild. Thus only one patient had a paresis, a fact which should be compared with the study by Holmgren *et al.* (5 These workers noted a frequency of flaccid paralysis in about 25 per cent of their patients in Sweden. The other clinical features of the Kumlinge disease correspond fairly well with those reported by Holmgren *et al.* (5 The convalescence however was shorter and more uneventful in the present series than among the patients in Sweden.

The relatively mild character of Kumlinge disease is also emphasized by the fact that the mortality in our material is nil. The same is true of the 30 virologically confirmed cases from Sweden presented by Holmgren *et al.* (5) and seems to be true also of the suspected cases on Bornholm in Denmark (3) This agrees well with the earlier description of diphasic tick borne meningo-encephalitis in the north-western U.S.S.R. by Smorodintsev (16)

In spite of its comparative mildness, Kumlinge disease is still an important cause of temporary disability among the population of Åland The patients who seek medical treatment represent only a fraction of those infected, a fact which is well known among the physicians practicing in the province. For this reason, prophylaxis against the disease is highly desirable either by eradication of the ticks or by active immunization of the population.

Summary

Of 27 patients with aseptic meningitis or meningo-encephalitis and antibodies to tick-borne encephalitis virus, 18 were regarded as virologically verified cases of the diphasic tick-borne meningo-encephalitis that occurs in the Åland Islands, *i.e.* of Kumlinge disease. The clinical and epidemiological features of these cases are described.

The diagnosis was based on a fourfold or greater increase in complement-fixing and/or haemagglutination-inhibiting antibodies. In 2 cases it was further confirmed by isolation of the virus from the acute

phase blood. The complement fixation test seems to be the method of choice for routine diagnosis of the disease.

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Symptomatology and Prognosis of Abdominal Aortic Aneurysm

By

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Aneurysmal dilatation of the aorta was a disease entity well known to the earlier clinicians. Syphilitic aortitis, however received most attention, since — especially in the thoracic aorta — it used to be the commonest aetiological factor Syphilitic aortitis is rarely encountered today Atheroma of the abdominal aorta seems to have taken its place.

The aneurysmal dilatation is caused by disintegration of the elastic fibres in the aortic wall Syphilitic aortitis produces massive destruction in the wall compared to the more diffuse changes of arteriosclerosis. The rhythmical dilatation of the pulsating aorta exerts mechanical stress on the injured wall, causing continuous microdestruction of the disintegrating elastic tissue. The interaction between the dilating pulse wave and the site size and severity of the intramural lesion gives aneurysms their various macroanatomical forms. Syphilitic aneurysms have been described as saccular and atheromatous ones as fusiform (4)

Recent progress in vascular surgery has created new interest in the subject and the diagnosis of an abdominal aortic aneurysm is no longer an academic curiosity Resection of aneurysms was introduced by Dubost *et al.* (8) In 1957 DeBakey *et al.* presented an analysis of 313 cases of surgical resection with encouraging results (7) With greater surgical experience and careful selection of patients a reduction in operative mortality to five per cent has recently been achieved (16)

The discouraging prognosis of untreated aneurysm as compared with successful surgery imposes upon the physician a compelling obligation to make an early diagnosis and a critical evaluation of the patient as a surgical risk. Although diagnosis previous to rupture is desirable, aneurysmal rupture is seldom immediately fatal and time is frequently available for diagnosis and surgery The diagnostic difficulties presented by the rupture of a previously unsuspected aortic aneurysm

may however at times be perplexing. The complication is highly acute and loss of time may be disastrous. However correct diagnosis is far from difficult, if the possibility is kept in mind. Thus, the symptomatology of these lesions must be clearly defined. We have attempted to describe the general symptomatology, the syndrome of aneurysmal rupture and the prognosis of the aortic aneurysms.

Material

The present series of abdominal aortic aneurysms was collected from the hospital files covering the fifteen-year period 1947–1962. The total number of cases is 36, of which 22 were discovered in the last five-year period. In 28 of these cases the outcome was fatal during the mentioned time of observation. Of the fatal cases 14 expired because of rupture of the aneurysm. The rupture was established or verified at autopsy. In nine cases with fatal outcome of other aetiology the presence of an abdominal aortic aneurysm was likewise established or verified at autopsy. The total number of autopsies is 23. In these 23 cases, the aneurysm was discovered incidentally at autopsy in seven, in one of which death was due to an unrelated disease. The cause of death in the other six cases was aneurysmal rupture. In the surviving eight cases rupture has not occurred. In two of the non-ruptured cases, where autopsy was not performed, death occurred suddenly. The course of events strongly suggested fatal rupture, but the exact cause of death was never ascertained. We have placed them among the non-ruptured cases. Consequently this group consists of 22 cases. Surgical resection was not attempted in the reported cases. The criteria for diagnosis are presented in Table I.

Table I. Criteria for the diagnosis in 36 cases of abdominal aneurysm.

I.	Clinical findings only		6 cases
II	Clinical findings verified		23
	A. by a) X-ray examinations	6	"
	b) including aortography	1	"
	B. by autopsy	16	
III	Incidental autopsy findings only		7
	Total		36 cases

Clinical Findings

Sex and age. The series consists of 24 men and 12 women. The average age at the time of the initial diagnosis, whether clinical or incidental at autopsy was 70 years. The youngest was a man of 56 and the oldest a woman of 85. All the patients over 80 were women. Half of the cases were diagnosed in their seventh decade. About a third of them were in their eighth decade. The average age in the group diagnosed incidentally at autopsy was identical with that of the material as a whole. The age distribution in two groups was similar. The sex and age distribution in the whole series is presented in Table II.

Table III shows the sex and age distribution of 14 cases of fatal rupture. The average age is identical with that of the group as a whole. The frequency of aneurysms in males compared with females is 2 to 1 in the whole series, while it is about 3.7 to 1 in the ruptured cases.

Signs and symptoms before rupture. Signs and symptoms of 29 cases before rupture are presented in Table IV. Seven ruptured cases, where no history prior to rupture was available, are excluded from the table.

Pain was present in 19 cases (65 %). In the ruptured cases pain preceding rupture was encountered more frequently (86 %). As a rule the pain was abdominal (62 %). However back pain was a frequent additional feature among the ruptured cases. Radiation of pain down to the lower extremities was noted in four cases. Furthermore, pain referred to the groin, thigh or renal region was mentioned in three additional cases.

Gastrointestinal symptoms occurred in 17 cases (59 %). Nausea and vomiting

was the leading feature. Eight patients were simultaneously bothered by obstipation. Genito-urinary symptoms and signs were scanty (21 %). They consisted of vague dysuria, proteinuria and haematuria.

Positive physical signs were present in 25 cases (86 %). A pulsatile abdominal tumour was noted in 15 cases (65 %). Only two of them were later complicated by rupture. A non-pulsatile tumour was found in only three cases. Tenderness was encountered in 13 cases (45 %) and muscular defence in only four (14 %). These signs were equally distributed between the ruptured and non-ruptured cases.

Symptoms and signs after rupture. Presenting symptoms and signs, as well as some laboratory findings, are summarized in Table V. Information has not been available on all points. The frequency is calculated only from the number of cases in which the aspect concerned has been considered by the physician in charge.

In all cases the onset of the rupture was signalled by abdominal pain. In seven additional cases back pain occurred. The character of the pain was invariably

Table II. Age and sex distribution in 36 cases of abdominal aortic aneurysm.

Age yrs.	All cases		Incidental autopsy findings		Men		Women	
	No.	%	No.	%	No.	%	No.	%
41–50	—	0	—	0	—	0	—	0
51–60	3	8	1	14	2	8	1	8
61–70	18	50	3	43	13	54	5	42
71–80	12	34	2	29	9	38	3	25
81–	3	8	1	14	—	0	3	25
Total	36	100	7	100	24	100	12	100
Average age	70		70		69		72	

Table III. Age and sex distribution in 14 cases of abdominal aortic aneurysm with fatal rupture.

Age	All ruptured cases		Men		Women
	No.		No.	%	N
51–60	1	7	1	9	—
61–70	7	50	6	55	1
71–80	5	36	4	36	1
81–	1	7		0	1
Total	14	100	11	100	3
Average age	70.5		69		74

described as continuous and usually severe. Only one patient regarded his pain as mild. In five patients the onset of pain was sudden, while in seven it was gradually increasing.

Pain was accompanied by nausea or vomiting in less than half the cases. Five patients complained of obstipation and diarrhoea occurred in one case.

Some kind of genito-urinary symptoms or positive urinalysis was noted in nine cases. Oliguria-anuria occurred in six cases. Dysuria was noted in one case. In eight cases proteinuria and/or haematuria was mentioned.

Abdominal physical signs accompanying rupture were present in all cases. There was a definite, pulsatile abdominal mass in slightly less than half the cases. A non-pulsatile mass was palpable in five cases. A palpable tumour was absent in only three cases. Tenderness, greatest over the mass, was observed in all but three cases. Muscular defence was an infrequent sign (four cases). Distension, although frequently reported by others, was only encountered in two cases.

Shock, excluding terminal shock before death, occurred in all but three cases. The duration of the shock varied from

Table IV. Symptoms and signs before the rupture in 29 cases of abdominal aortic aneurysm[1]

Symptoms or signs	All cases No.	%	Non-ruptured cases No.	%	Ruptured cases No.	%
I. Pain	19	66	13	59	6	86
site of pain:						
abdomen	18	62	13	59	5	71
back	7	24	3	14	4	57
lower extremity	4	14	4	18	0	0
other	3	10	2	9	1	14
None	10	35	9	41	1	14
II. Gastrointestinal						
symptoms:	17	59	14	64	3	43
nausea	9	31	7	32	2	29
vomiting	8	28	6	27	2	29
diarrhoea	2	7	2	9	0	0
obstipation	8	28	6	27	2	29
III. Genitourinary						
symptoms	6	21	5	23	1	14
IV. Physical						
signs:	25	86	20	91	5	71
pulsatile mass	19	65	17	77	2	29
non-pulsatile mass	3	10	1	5	2	29
tenderness	13	45	10	46	3	43
muscular defence	4	14	3	14	1	14
Total	29	100	22	100	7	100

[1] Seven ruptured cases, where no history was obtained, are excluded from the table.

Table 1 Presenting symptoms, signs and some laboratory findings after rupture.

Record No	Age and sex	Survival time after rupture	Character of pain: Constant	Character of pain: Intermittent	Character of pain: Sudden	Character of pain: Gradually increasing	Character of pain: Severe	Character of pain: Mild	Site of pain: Abdominal region[1]	Site of pain: Back	Gastro-intestinal symptoms: Nausea	Gastro-intestinal symptoms: Vomiting	Gastro-intestinal symptoms: Diarrhoea	Gastro-intestinal symptoms: Obstipation	Genito-urinary symptoms: Anuria	Genito-urinary symptoms: Oliguria	Genito-urinary symptoms: Dysuria	Urinalysis: Proteinuria	Urinalysis: Haematuria	Abdominal physical signs: Pulsatile mass	Abdominal physical signs: Non-pulsatile mass	Abdominal physical signs: Tenderness	Abdominal physical signs: Muscular defence	Abdominal physical signs: Distension	Duration of shock	Laboratory findings: Haemoglobin g % Maximum	Laboratory findings: Haemoglobin g % Minimum	Laboratory findings: Leucocyte count 1000 cells/mm³	Laboratory findings: Sedimentation rate	Laboratory findings: Plasma creatinine mg/100ml	Laboratory findings: Melaena
1962/47	56 ♂	1 day							A VI	−	+	−	−		−		−	+	−	−	+	−	−	−	−						
677/52	62 ♂	0.5 day	+		+		+		A	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	12h						
1471/53	69 ♀	3 days	+		+				A I	+	+	+	+	−	+	−	−	+	+	+	−				1 day	13.4	11.5	17.9			
585/53	73 ♂	0.75 day	+			+	+		A I	+	+	+	−	−	−	−	−	−	−	+	−	+	+	−	12h						
446/53	69 ♂	1.5 days	+			+	+		A IV	+	+	+	−	+	−	−	+	+	−	+	−	−	−	−	−	13.8		5.6	10		
2071/58	67 ♀	5 hrs	+		+		+		A I, III, V	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	5h			13.1			
3091/58	75	16 days	+		+		+		A II	+			−	−	+	−	−	+	−	−	+	−	−	−	−	9.8	9.7	13.4	47		
2108/59	63	23 days	+			+	+		A VI	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	1 day	12.5	8.9	20.5	141		+
3152/59	76 ♂	1 day	+			+	+		A VI, IX	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	18h		7.8	21.6			
1785/60	79 ♀	11 days	+		+		+		A IX	−	−	−	−	+	−	−	−	−	−	−	+	+	−	−		8.7	0.7	10.3	61	(NPN = [illegible])	
70/61	66 ♂	6 days		+		+		+	A VII, VIII	−	+	−	−	−	+	−	−	+	+	−	+				22h	10.4	8.6	16.7	81	7.5	
2580/61	81	2.5 days							A I	+	−	−	−	+	+	−	−	+	−	−	−	+	+	−	15h	11.1		21.4	44	3.75	
2140/61	73 ♂	1 day	+			+	+							+						−	+	+	−	−	1 day						
1591/61	69 ♂	3 day	+			+	+		A VII	+	−	−	−	−	+	−	−	+	−	+	−	+	−	−	5d	13.9	9.7	30.8	12	5.90	+
%	14	AVERAGE 4.5 DAYS	11/12	1/12	5/12	7/12	11/12	1/12	13/13	7/13	5/13	3/13	1/14	5/14	5/13	1/13	1/13	8/13	2/13	6/14	5/13	8/12	4/12	2/12				9/10			
			92	8	42	58	92	8	100	54	38	23	7	36	39	8	8	62	15	43	36	67	33	17				90			

AI – AIX = abdominal regions: right hypochondrium, epigastrium, left hypochondrium, right lumbar, umbilical, left lumbar, right iliac, hypogastrium, left iliac.

five hours to three days. Where haemoglobin was determined there was a rapid fall due to internal haemorrhage. There was no significant correlation between the duration of the shock and the length of survival after rupture. The longest survival was 25 days and the shortest five hours. The average length of survival in the series was 5.5 days.

Marked leucocytosis was found in all but one case. The size of the haematoma in this case was the same as in the other cases. Acute azotaemia was noted in two cases. One of the azotaemic patients had chronic pyelonephritis. This azotaemia may be explained by chronic renal failure. Case No. 1785/60K had a normal urine output but raised NPN probably because of senile changes in the kidneys. In three other cases with anuria-oliguria the plasma creatinine was not determined. In the other cases azotaemia was apparently not looked for because of sufficient urine flow. Melaena was diagnosed in two out of five cases.

Autopsy Findings

The incidence of abdominal aortic aneurysm at autopsy for the whole period is 0.48 % (23 cases in 4792 autopsies) and that of ruptured aneurysms 0.29 %. For the period 1947—56 it is 0.28 % (9 cases in 3167 autopsies) and 0.16 % (5 cases) respectively while in the period 1957—62 it is 0.86 % (14 cases in 1625 autopsies) and 0.56 % (9 cases) respectively. Thus these lesions are now found at autopsies about three times as often as was the case in the earlier period. The frequency of ruptured aneurysm at autopsy has increased three and a half times during the period concerned.

In 19 of the autopsied cases the location of the aneurysm was clearly recorded. The site of the aneurysm in these cases is presented in Table VI. In these cases the total number of aneurysms was 22, three cases having two aneurysms. The aneurysms in this series were most frequently located below the renal arteries. Only in four cases were they situated above the renal arteries, while in an equal number of cases the aneurysm arose in the common iliac vessels or at the aortic bifurcation.

The autopsy findings in the 14 cases of ruptured aneurysms are presented in Table VII. Nine out of eleven aneurysms were saccular in form, the rest being fusiform. This division is, however, not strictly accurate, owing to the difficulty of describing the shape of a ruptured aneurysm. Dissection of the aortic wall in connexion with the aneurysms was found in two cases. The size of the haematoma measured from one to two litres and the haematoma was in all cases limited to the retroperitoneal space. The haematoma extended to the kidney in ten out of 12 cases. The kidney was displaced by the haematoma in only five of these cases.

Table VI. Location of the aneurysm in 19 autopsied cases[1]

Site of aneurysm	No.	%
Above the renal arteries	4	18
Below the renal arteries	14	64
Aortic bifurcation or the common iliac vessels	4	18
Total	22	100

[1] In three cases two aneurysms were found. In four cases the pathologist had not mentioned the exact site of the aneurysm. They are excluded from the table.

Discussion and Comments

With the increasing life-span of the general population arteriosclerotic aneurysms of the aorta have become more frequent. As a result of successful antiluetic therapy syphilitic aneurysms are rapidly disappearing The over-all incidence of abdominal aortic aneurysms seen at autopsy in this hospital was 0 48 % for the whole period. In our series the incidence at autopsy for aortic aneurysms increased by about three-fold during the period 1947—1962 and even slightly more for cases terminating in rupture. None of these aneurysms were regarded as syphilitic in origin. In the beginning of this century up to 70 % of abdominal aortic aneurysms were of syphilitic origin in a Boston series, while in a large series reported from the Mayo Clinic 95 % were arteriosclerotic (14,9) The increasing incidence of aortic aneurysms has

Table VII Necropsy finding in 14 cases of ruptured aneurysm of the abdominal aorta.

Record no	Site of the aneurysm	Macroanatomic form	Dissection of the wall	Haematoma		Displacement of kidney
				site	size	
1962/47		saccular	———			———
677/52	just below the renal arteries	saccular	———		1 5 l	———
1471/53		saccular	———			
585/54			———		1 5 l	———
446/55		fusiform	———		1 5 l	———
2071/58			———		1 5 l	———
3091/58	5 cm below the diaphragm	fusiform	———		1 5 l	both
2108/59	just below the renal arteries	saccular	+		1 5 l	left
3152/59		saccular	———		1 5 l	———
K1785/60	1) left common iliac artery 2) above the aortic bifurcation	saccular	———		1 0 l	left
26/61	just above the renal arteries	saccular	———		1 5 l	———
2580/61	just below the renal arteries	fusiform	+		2 l	right
2140/61		saccular	———		1 l	———
2591/62		saccular	———		1 l	———

Table VIII Additional autopsy findings and the clinical diagnosis in 28 fatal cases of abdominal aortic aneurysm.

Record number	Additional findings at necropsy	Aneurysmal rupture main cause of death
I. Ruptured cases		
1962/47	Pulmonary emphysema	yes
677/52	None	yes
1471/53	Cardiac hypertrophy, pulmonary oedema, right renal cyst	yes
585/54	Nephrosclerosis	yes
446/55	Cardiac hypertrophy, arteriosclerosis	yes
2071/58	Myocardosis, hepatosis, nephrosclerosis	yes
3091/58	Pulmonary embolism, thrombosis of the inferior vena cava	yes
3152/59	Pulmonary emphysema, chronic prostatitis with hyperplasia, haemangioma, chronic pyelonephritis	yes
2108/59	Myofibrosis of the heart, pancreatitis	yes
1785/60	Senile changes in the kidneys	yes
26/61	Cholelithiasis, parenchymatous degeneration of the right kidney	yes
2580/61	Hypertrophy of the left cardiac ventricle, cholelithiasis, chronic pyelonephritis	yes
2140/61	Pulmonary emphysema	yes
2591/61	Myofibrosis of the heart, hepatosis, nodular goitre	yes
II. Unruptured cases		
1735/52	Recent thrombosis of the coronary artery	no
2373/55	Cardiac hypertrophy, nephrosclerosis	no
2677/55	Hydrothorax, pulmonary congestion, hepatitis, cardiac infarction, recent pyelitis	no
2583/58	Pulmonary emphysema, chronic pyelonephritis, adenoma of the left adrenal gland	no
2692/59	Recent infarction and myofibrosis of the heart, atherosclerosis and stenosis of the coronaries, benign tumour of the right kidney	no
1813/52	Cerebral *infarction, myodegeneration of the heart*	no
2800/61	Subserosal haematoma of the urinary bladder	no
4142/60	Cholangitis	no
833/61	Grave myofibrosis of the heart and atherosclerosis of the coronaries, blood in the gastro-intestinal tract	no

Record number	Additional diagnoses in non-autopsied cases	Aneurysmal rupture probably main cause of death
1527/54	Abdominal tumour, secondary anaemia	yes
923/54	Cerebral haemorrhage, hypertension	no
2468/54	Pulmonary carcinoma, arteriosclerosis	no
255/59	Cardiac sclerosis, symptomatic anaemia. Atrial fibrillation, acute pyelocystitis	no
215/61	Arteriosclerosis, atrial fibrillation, congestive heart failure, arterial embolism of the left calf, pyelonephritis	yes

been emphasized by Blakemore *et al.* and Brindley *et al.* (2,3) Magnilla *et al.* (12) observed an incidence of 0.5 % in 6 000 autopsies and Barrat Boyes (1) reported an incidence of 0 66 % with a three fold increase over the period 1939—1956. These figures correspond with our findings.

It is well established that the great majority of arteriosclerotic abdominal aneurysms arise from the aorta below the level of the renal arteries. In our series only eighteen per cent arose above the renal arteries. Furthermore, arteriosclerotic aneurysms occur in the higher age groups. The average age is reported to be over 60 years (1) In men they have been reported to arise t as early an age as 45 years (1) The syphilitic neurysms, however had an age range from 20 to 50 years (13) The average age in our series is 70 years. These facts confirm the opinion that the aneurysms in our series were of arteriosclerotic origin.

Syphilitic abdominal aneurysms usually arise above the renal arteries, causing root pain due to erosion of vertebral bodies and dyspepsia while enlarging forward against the stomach. In Kampmeier's series such aneurysms caused pain in 90 % (11) Arteriosclerotic aneurysms, arising mostly below the renal arteries, are more silent. Barrat Boyes (1) noted pain before rupture in only 46 %. The range in our series is from 59 % for non-ruptured aneurysms to 86 % for aneurysms later complicated by rupture. However pain from accompanying disease cannot be strictly excluded in our series. Our figures therefore tend to be slightly on the high side. Pain caused by an arteriosclerotic aneurysm is most frequently located in the abdomen. Back pain is definitely an alarming sign suggesting incipient rupture. The mechanism behind the pain is probably minor retroperitoneal extravasation of blood. Bizarre symptoms may at times occur due to compression of the ureters or the renal vessels, thus simulating urological or renal disease (17 15) Compression of the duodenum has even been described (18)

The presence of palpatory signs prior to the rupture depends on the size of the aneurysm and the obesity of the patient. An aneurysm measuring less than 4 cm in diameter is hardly palpable. A pulsatile abdominal tumour is astonishingly often bsent, although with greater care ane urysms would surely be palpated more often. Some kind of palpable mass was found in 22 cases of our series.

After rupture the difficulty of palpating the aneurysm may even increase due to abdominal distension and muscular de fence as well as decreasing tension within the aneurysmal sac. Later when the retroperitoneal haematoma has at tained considerable dimensions, it may easily be felt. Rigidity of the abdominal muscles, which accompanies so many abdominal emergencies, is a less frequent sign in th rupture syndrome We encountered it in four out of 12 cases. Even shock is not a feature of the rupture syndrome and the illness frequently proceeds less dramatically than would be expected. Death may be delayed for weeks.

Laboratory findings give little support to the diagnosis. A rapid fall of the haemoglobin corresponds to an increasing haematoma. Marked leucocytosis occurred in several of our cases, although it has

been reported to be moderate by others (1) Radiological examination has been recommended even in cases of rupture. No retroperitoneal haematoma could be demonstrated in our cases. In non-ruptured aneurysms, however plain X-ray films were of considerable value.

Other abdominal emergencies may resemble an acute rupture. Acute pancreatitis and mesenteric vascular occlusion presenting with severe pain and shock of varying degree but only minor peritoneal irritation, are mentioned as points of differential diagnosis. Elevated serum levels of pancreatic enzymes have, however been observed in connexion with rupture. Melaena, often seen in mesenteric vascular occlusion was observed in some of our cases. These signs consequently do not exclude rupture.

The unfavourable prognosis in patients with arteriosclerotic aneurysm has frequently been emphasized but the conclusion to be drawn from this is often ignored. With the introduction of successful surgical therapy this attitude can no longer be defended. Our series may serve as an illustration. Additional findings at autopsy in 23 cases are presented in Table VIII All the patients had received medical attention for some other disease. In fourteen cases (14 out of 23 deaths, 61 %) however the aneurysm turned out to be cause of death. In the whole series of 36 cases sixteen can be regarded as having terminated in a fatal rupture. This gives a mortality of 45 % None of the ruptured cases survived with so-called conservative management.

In a large series Gliedman *et al.* have observed that 50 % of patients with abdominal aortic aneurysm were dead within one month of detectable signs, 75 % were dead within six months and 80 % had expired in less than one year(10) Our series is too small for such an estimation but it is compatible with these observations. The prognosis following rupture of the aneurysm is even graver A mortality of 50 % within 24 hours and 90 % within six days has been mentioned (15) Sudden death is seldom caused by a rupture. Copping (6) found peaks of death at eight hours and two days after rupture. The shortest survival time in our series is five hours and the majority of deaths (71 %) occurred within the first three days. However three cases survived for 11—25 days. This confirms previous experience that ruptured abdominal aneurysms are not necessarily immediately fatal. Time for prompt surgical intervention is at times amply available. Even a rupture constitutes no contraindication for surgery Cooley has reported that 11 out of 18 patients with a rupture survived surgical resection. There is little doubt that, excision should be undertaken as soon as possible after the diagnosis of a rupture is established (5) In conclusion it may be asserted that these figures concerning non-ruptured as well as ruptured aneurysms, when compared with the low operative mortality five per cent, of competent surgery in correctly selected cases of unruptured aneurysms of the abdominal aorta, constitutes an urgent call for a more active attitude

Summary

Arteriosclerotic aneurysm of the abdominal aorta is a disease of old age occurring more frequently among men The incidence of arteriosclerotic aneurysms is

markedly increasing, owing to the increasing life-span. Usually arising below the renal arteries these aneurysms are comparatively symptomless. The appearance of signs and symptoms indicates progressing dilatation and a fatal issue. Back pain suggests incipient rupture. The diagnosis is frequently easy if the condition is kept in mind. Presenting signs and symptoms are often illustrative. Atypical cases occur but with X ray examination and occasionally aortography the diagnosis is rarely missed. Because of the catastrophic nature and lethal outcome of a rupture the correct diagnosis should be made early. Rupture, however is not immediately fatal and time for rapid surgery is often available. The astonishingly low operative mortality of competent surgery has totally displaced conservative management.

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From the Kustaankartano Home for the Aged, Helsinki, Finland.

Laxative Effect of Sennoside A and Placebo in a Double-blind Cross-over Test

By

S. G. Jokipii and Gustaf Östling

Functional constipation represents a troublesome problem and an administrative and procedural burden for those institutions concerned with geriatric care.

The term functional constipation as used here may be defined as any or all of the following complaints without any known organic cause: Failure of the bowels to move with the customary frequency, sensation of incomplete bowel evacuation, and passage of sparse, hard masses. Functional constipation is a condition which is tolerated fairly well and which often subsides spontaneously within a short time. The treatment must naturally be less dangerous and difficult than the complaint itself.

Because of the nature of functional constipation it is obviously difficult to assess the effect of mild laxatives. Experiences gained in general clinical practice are unreliable because account has to be taken of both spontaneous correction and the placebo effect. Opinions regarding the significance of the last-named factor vary considerably. Clauser *et al.* (1) and Haas *et al.* (2) obtained such poor results with placebo preparations in double-blind tests in functional constipation that in their opinion the double-blind placebo technique need not be used. Zuspan (9) on the other hand, with double-blind treatment of postpartum constipation achieved the same result with the placebo as with the effective substance.

In the present double-blind cross-over placebo tests we have endeavoured to imitate the conditions under which laxatives are usually employed with a view to forming an opinion of the rôle of the placebo effect in the treatment of functional constipation at a home for the aged. Moreover a comparison was made in these tests between the laxative effect of 25 and 50 mg Sennoside A. In preliminary clinical tests we had demonstrated that both 25 and 50 mg Sennoside A have a good effect similar to that of other gen

erally used laxatives of the same type (3)

Sennosides A and B were isolated from the senna drug in 1941 by Stoll *et al.* (4) Pharmacological investigations (5 6 7) produced the impression that Sennoside A was a milder laxative with less risk of spasms and other side-effects than Sennoside B. Clinical tests with pure Sennoside A as a laxative have not been published before to our knowledge. On the other hand, combined preparations containing equal parts of Sennosides A and B have been widely used in clinical practice.

Materials and Methods

The tests were carried out in 9 wards at municipal home for the aged with total of 634 beds for healthy old people. Of the 200 patients treated 175 were female The patients ages ranged from 62 to 88 years.

The tests made use of crystalline calcium salt of Sennoside A with an equivalent weight of 449 and melting point of 225—235 C (decomposition) In paper chromatography and in counter current distribution the substance was pure and uniform.

The double-blind cross-over test was made in 2 different series of investigations. First the effect of 50 mg Sennoside A was compared to placebo (50 mg Sennoside A series) and 4 months later 25 mg Sennoside A with placebo (25 mg Sennoside A series)

50 mg Sennoside A series

Tablets with 25 mg Sennoside A and placebo tablets containing inert tablet mass were prepared. All tablets were tasteless and of the same colour size and shape. Six placebo tablets and six Sennoside, tablets were reserved for each patient. Each series of tablets had its own code and the system was worked out in such manner that half the patients, selected at random, were first treated with Sennoside the other half receiving placebo first. The code was opened only after all the patients had been treated and the results assessed.

The routine at the home for the aged was such tha the ward sisters administered two tablets of some suitable laxative as preliminary measure in the treatment of functional constipation. At the beginning of the investigation the nurses were instructed to administer the test tablets in the usual manner and not to tell the patients that study was in progress. The nurses were not told that one series of the tablets was placebo; instead they were given the impression that two new laxatives were being compared to each other Furthermore the nurses were told not to alter their accustomed attitude towards the patients who received the test preparations by subjecting them, for instance, to more searching inquiry than usual about the results of the treatment.

All those old people who during the test period complained of constipation were given as usual tw tablets containing either 50 mg Sennoside A or placebo. If the patient returned on the following day because the first dose had failed to have any effect, another two tablets of the same kind were given. If the condition failed to respond, two more tablets were given on the third day In those cases in which the third dose failed to be effective within maximum of 12 hours, the constipation was treated in some other suitable way If an old person who had been treated in such manner again came and complained of constipation, the other kind of tablets was given in exactly the same way The patients were not asked more than usual about the effect of the tablets. The decisive criterion of the effect of the tablets was whether the patient came and asked for further treatment or not. Owing to this arrangement some of the patients, depending on the effect, received only one dose of two tablets, some others tw doses and the remainder three doses of the test tablets.

25 mg Sennoside A series

Four months after the termination of the 50 mg Sennoside A series the tests were continued in exactly the same manner with tablets of the same external appearance. This time the Sennoside tablets contained 12.5 mg each. The dose administered was consequently 25 mg Sennoside A at one time.

The nursing staff was told that the previous experiments had revealed difference in the effects of the two laxatives, but that the tests must be continued in the same manner to make it possible to obtain better idea of the significance of the difference.

Results

Tables I and II summarize the results of the 25 mg and 50 mg Sennoside A series, respectively Both series comprised 100 treated patients. In the 25 mg series the treatment could be carried out according to plan in 91 cases and in the 50 mg series in 97 cases. In both Tables, group 1 comprises those patients whose constipation was treated for the first time with Sennoside and for the second time with placebo while group 2 consists of patients who were treated in the reverse order The results of treatment have been entered in the Tables with the symbol + which signifies that constipation was cleared up or with the symbol 0, which means that the medication failed to have any effect.

Table III was compiled from the results of Tables I and II and shows how many patients had their constipation corrected with 1 2 and 3 doses of these tablets. It emerges from the combined result of both series that 1 dose of Sennoside A had a better effect than 1 dose of placebo ($P<0.001$) The difference between 3 doses of Sennoside and 3 doses of placebo was also highly significant ($P<0.001$) On the other hand, no difference could be demonstrated between the effects of 25 and 50 mg Sennoside A.

Table I. Survey of the results in the 25 mg Sennoside A series (legend, see text)

Group 1							Group 2						
25 mg Sennoside Day of treatment			Placebo Day of treatment			No. of patients	Placebo Day of treatment			25 mg Sennoside Day of treatment			No. of patients
1st	2nd	3rd	1st	2nd	3rd		1st	2nd	3rd	1st	2nd	3rd	
+			+			21	+			+			15
+			0	+		5	+			0	+		1
+			0	0	+	1	+			0	0	0	1
+			0	0	0	8	0	+		+			4
0	+		+			1	0	+		0	+		3
0	+		0	0	+	1	0	+		0	0	+	1
0	+		0	0	0	1	0	+		0	0	0	2
0	0	+	+			1	0	0	+	+			1
0	0	+	0	+		1	0	0	+	0	+		1
0	0	+	0	0	+	1	0	0	+	0	0	0	1
0	0	+	0	0	0	2	0	0	0	+			7
0	0	0	0	+		1	0	0	0	0	+		1
0	0	0	0	0	+	1	0	0	0	0	0	+	4
0	0	0	0	0	0	1	0	0	0	0	0	0	3
						46							45
+						1	+						1
+			0	0		1	0	+					1
0	+					1	0						1
0			+			1	0	0	0				1
							0	0	0	0	0		1
Total						50	Total						50

Table II Survey of the results in the 50 mg Sennoside A series (legend, see text)

Group 1							Group 2						
50 mg Sennoside Day of treatment			Placebo Day of treatment			No. of patients	Placebo Day of treatment			50 mg Sennoside Day of treatment			No. of patients
1st	2nd	3rd	1st	2nd	3rd		1st	2nd	3rd	1st	2nd	3rd	
+			+			19	+			+			16
+			0	+		4	+			0	+		6
+			0	0	+	2	+			0	0	0	1
+			0	0	0	10	0	+		+			7
0	+		+			4	0	+		0	0	0	1
0	+		0	+		1	0	0	+	+			4
0	+		0	0	+	1	0	0	+	0	0	0	1
0	+		0	0	0	2	0	0	0	+			9
0	0	+	+			2	0	0	0	0	+		1
0	0	+	0	0	0	2	0	0	0	0	0	0	2
0	0	0	+			1							
0	0	0	0	0	0	1							
						49							48
+			0			1	0	0		0			1
							0	0	0	0			1
Total						50	Total						50

le III. Laxative effect of Sennoside A and placebo in double-blind cross-over tests.

	Dosage	Constipation cleared up	Dosage	Constipation cleared
ng Senno-	1 × 25 mg	62 patients (68%)	1× placebo	40 patients (44
A series,	2 ×	9	2×	17
patients	3 × „	10	3×	7 „
		Total 81 „ (89%)		Total 64 „ (70
ng Senno-	1 × 50 mg	71 (73%)	1 × „	49 (51
A series,	2 ×	15	2 × „	13 „
patients	3 × „	4 „	3 ×	8 „
		Total 90 „ (93%)		Total 70 (72
al results	1 dose Sennoside	133[1] (71%)	1 dose placebo	89[2] (47
both series,	3 doses Sennoside	171 „ (91%)	3 doses placebo	134 (71
patients				

fference highly significant ($\chi^2 = 20.4$ $P<0.001$)
„ ($\chi^2 = 22.5$ $P<0.001$)

Table IV is composed in such a way that the effect of 1 dose of Sennoside on patients refractory to 1 dose of placebo can be compared with the effect of 1 dose of placebo on patients refractory to 1 dose Sennoside A. In this manner Sen-

Table IV Laxative effect of 1 dose of Sennoside A and 1 dose placebo on patients refractory to 1 dose placebo and 1 dose Sennoside, respectively, in double-blind cross-over tests.

	No. of patients refractory to 1 dose placebo	Constipation corrected with 1 dose Sennoside		No. of patients refractory to 1 dose Sennoside	Constipation corrected with 1 dose placebo	
25 mg Sennoside A series	51	26[1] patients	(51 %)	29	4 patients	(14 %)
50 mg Sennoside A series	48	36[2] "	(75 %)	26	14[2]	(54 %)
Total of both series	99	62[1]	(63%)	55	18[3] "	(30%)

[1] Difference highly significant ($\chi^2 = 13.79$ $P<0.001$)
[2] significant ($\chi^2 = 5.11$ $P<0.05$)
[3] " ($\chi^2 = 8.26$ $P<0.01$)

Table V Laxative effect of 1 dose of Sennoside A and 1 dose placebo on patients refractory to 3 doses placebo and 3 doses Sennoside respectively in double-blind cross-over tests.

	No. of patients refractory to 3 doses placebo	Constipation corrected with 1 dose Sennoside		No. of patients refractory to 3 doses Sennoside	Constipation corrected with 1 dose placebo	
25 mg Sennoside A series	27	15 patients	(56%)	10	1 patient	(10%)
50 mg Sennoside A series	27	19	(70%)	7	2 patients	(29%)
Total of both series	54	34[1]	(63%)	17	3[1] "	(18%)

Difference significant ($\chi^2 = 8.89$ $P<0.01$)

Table VI Side-effects.

	Medication	Nausea	Griping	Pain
25 mg Sennoside A series	25 mg Sennoside	2 pat.	1 pat.	0 pat.
	Placebo	3	0 "	2
50 mg Sennoside A series	50 mg Sennoside	2	1 "	1
	Placebo	7	5 "	0

noside A was found on analysis to be more effective than placebo ($P<0.001$) The effect of 50 mg was better than that of 25 mg Sennoside ($P<0.05$) Moreover placebo had a significantly better effect in the 50 mg than in the 25 mg series ($P<0.01$)

Table V presents a comparison of the effect of 1 dose of Sennoside on those patients who were refractory to 3 doses of placebo and the effect of 1 dose of placebo on those patients who were refractory to 3 doses of Sennoside. A significantly better effect could be demonstrated with Sennoside ($P<0.01$) The data in this Table do not warrant the conclusion that there is a difference between the effects of 25 and 50 mg of Sennoside A.

Too potent an effect in the form of diarrhoea was produced in one patient with 25 mg Sennoside and none with placebo in the 25 mg series, whereas in the 50 mg series 12 patients had diarrhoea from 50 mg Sennoside and 3 from placebo. The difference in the incidence of diarrhoea between 25 and 50 mg Sennoside A is significant (Sign test, $P<0.01$)

Table VI shows the incidence of side-effects which the patients ascribed to the medication in the two test series.

Discussion

The results of the present study seen in Table III, show that approximately half the patients with functional constipation achieved bowel function with 1 dose of 2 placebo tablets. When the treatment was continued for 2—3 days a total of 70 % of the patients could be relieved of their complaint with placebo tablets. The observation that the placebo effect improved when placebo was administered several times consecutively indicates that a time factor and spontaneous correction of the constipation are included in the placebo effect.

In clinical examinations of drugs endeavours are made to eliminate the placebo effect as much as possible (2,8) In order to eliminate the time factor it is therefore reasonable, when studying the effect of laxatives on functional constipation to ascribe greater significance to the effect of the first administration of a laxative than to that of several repeated ones. If Table III is studied it will be noted that 1 dose of Sennoside had a better laxative effect than 1 dose of placebo ($P<0.001$) Another method to reduce the interfering influence of the placebo effect is to eliminate from the material so-called placebo reactors, that is to examine the effect of the active substance on those patients who did not respond to placebo Table IV shows that of 99 patients who were refractory to 1 dose of placebo (non placebo reactors) 63 % had their constipation corrected with Sennoside A. But there were also patients who were refractory to Sennoside and who reacted to placebo It is apparent from the Table that of 55 such patients 30 % had their constipation corrected with placebo. This naturally reduces the value of the observed Sennoside effect on patients refractory to placebo Nevertheless, Sennoside had a better effect on patients refractory to placebo than placebo had on patients refractory to Sennoside. The difference was highly significant and argues that Sennoside A had a better laxative effect than placebo.

Table V was constructed in the same

manner as Table IV on the assumption that patients who failed to react to 3 consecutive doses of placebo are surer non placebo reactors than those who did not react to only 1 dose of placebo Of 54 patients refractory to 3 doses of placebo and 17 patients refractory to 3 doses of Sennoside, 63 and 18 % reacted to 1 dose of Sennoside and 1 dose of placebo respectively Consequently even when analysed in this manner Sennoside A had a significantly better effect than placebo

It is obvious that one cannot completely exclude the placebo effect by the method of selecting in cross-over tests patients refractory to placebo, since in this study there were patients in whom constipation was cleared up with placebo although the laxative failed to produce any effect. One of the most important reasons is probably that two consecutive constipational episodes in one and the same person may be of very different degrees of severity

The second objective with this double-blind cross-over test was to investigate whether it was possible to distinguish between the effects of two different doses of the same preparation. In the preliminary tests (3) 25 and 50 mg Sennoside A had approximately the same effect. Nor does Table III which embraces th whole series of patients, reveal any difference between these two dosages. Table IV which comprises those patients who were refractory to 1 dose of placebo, shows that 50 mg had a better effect than 25 mg Sennoside ($P < 0.05$) No difference between these two dosages can be noted in Table V The statistical significance of the results in T bl IV for better effect of 50 mg than 25 mg Sennoside is weak, but it is strengthened by the observation that 50 mg Sennoside A had a more potent effect in the form of diarrhoea in 12 patients, whilst 25 mg produced diarrhoea in only 1 patient. The results of the tests may therefore be interpreted to indicate that 50 mg Sennoside has a somewhat more potent laxative effect than 25 mg Assuming the difference in the effects in Table IV to be real, it can be calculated that a series including approximately twice the number of patients would be necessary to make this difference significant ($P < 0.01$)

It is known that the active substance in double-blind placebo tests influences the effect of the placebo preparations (2 8) It is apparent from T ble IV that placebo tablets in the 50 mg Sennoside A series had a significantly ($P < 0.01$) better laxative effect than these same placebo tablets in the 25 mg Sennoside A series. In the former series there were, moreover 3 cases of diarrhoea with placebo, whereas no case of diarrhoea induced by placebo occurred in the latter series. It may furthermore be observed that the incidence of side-effects was highest with placebo in the 50 mg Sennoside A series (Table VI) Possibly these placebo effects in the 50 mg Sennoside A series may be interpreted as support for the view that 50 mg Sennoside A had a better effect than 25 mg

To summarize, it can be said that there was not a statistically significant difference in the laxative effect of 25 and 50 mg Sennoside in these old persons, who showed a high tendency to placebo reactions. Consequently the same method cannot be used for a comparison between two different laxatives with a mild effect,

unless a very great number of constipation episodes is studied. It should be possible, by modifying the method so that only the effect of single doses is examined, to carry out such an investigation at a large home for the aged.

This double-blind cross-over test was planned and could be carried out in such a way that it imitated as closely as possible the circumstances under which laxatives are used at the home concerned. The results obtained make it possible to draw certain conclusions about the administration of laxatives in such an institute. From the considerable effect of the placebo, the inference may be drawn that too much laxative is given in the wards and that it should be replaced by some other mode of treatment. On account of shortage of staff it is difficult to introduce a different therapy unless this is simpler than the administration of tablets. On the other hand, it should be regarded as a therapeutical-technical benefit that functional constipation in the old at a home for the aged reacts so well to placebo as to relieve 50 to 70 % of the patients of their complaint solely by administration of tablets as such. Moreover if the tablets contain a mild laxative substance without any side-effects, a laxative is obtained which, with a slight contribution in work by the nursing staff, can clear up the majority of cases of functional constipation. It would appear that Sennoside A tablets are well able to meet these requirements for a laxative.

Summary

It was found in a double-blind cross-over test at a home for the aged that 50 % of old persons with functional constipation had their complaint cleared up with 1 dose of placebo tablets. When the treatment was continued for 2—3 days a total of 70 % of the patients could be relieved of their complaint with placebo.

Sennoside A had a better laxative effect than placebo and was more effective in 50 mg than in 25 mg doses. The statistical significance of the difference between the effects of these two doses did not exceed the 95 per cent level but it was strengthened by the circumstance that diarrhoea occurred more frequently after 50 than after 25 mg Sennoside A.

Complaints about side-effects in the shape of nausea, griping and pain in the abdomen were rare and were noted somewhat more frequently after placebo than after Sennoside A.

Acknowledgements

We wish to thank the nursing staff of the Kustaankartano Home for the Aged for their valuable contribution to this study. We are further indebted to Oy Medica Ab, Helsinki, for the active substance Sennoside A and for the manufacture and codification of the test tablets.

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SUPPLEMENTUM 413

INFECTIOUS MONONUCLEOSIS

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By

JANNE PEJME

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INFECTIOUS MONONUCLEOSIS

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By

PEJME

ERRATA

Page 5 right column second paragraph line 3 for 5 read 55 line 5 for "19" read 17"

Page 30 left column third paragraph line 3 for slight read "slightly

Page 32 left column third paragraph from bottom line 2 delete "such

Page 38 left column line 8 for) mom read ymp-
.s

Page 39 left column the last sentence before the table should read "The material first dealt with in it entirety is then divided into sero-positive and sero-negative groups, into males and females and finally into adults and children (Tables 7 8)

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CONTENTS

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INTRODUCTION

Mononuclear cells of atypical character have long been observed in the peripheral blood of patients suffering from infectious mononucleosis. Such cells have since been found to be present in a number of other infectious diseases, in certain allergic states, in states of stress and finally even in apparently healthy individuals.

If it is accepted, as by the majority of authors, that infectious mononucleosis may be diagnosed in sero-negative cases as well, then such diagnoses must be solely clinical and haematological. A reliable guide for the clinical diagnosis of the disease has always been the particular occurrence of atypical mononuclear cells. However, since more recent experience has demonstrated that the atypical cells occur in a number of disorders and even in healthy persons, the demands set for the clinical diagnosis of infectious mononucleosis have thus been made more stringent. Nowadays better criteria than before are necessary to make such diagnosis absolute.

The present work, which comes from the Hospital for Infectious Diseases in Stockholm where infectious mononucleosis is diagnosed clinically and not merely serologically, gives an account of salient data on a material of patients suffering from this disease which was assembled during the years 1955—1956. Great importance has been attached to the haematological analysis with regard to the study of the white blood cells. The distinction between what is normal and what is atypical in the mononuclear cell elements must, to be able to make any claim to relevancy, be consistent. The observations included in this study were carried out by only two persons, who followed a common principle which is described in detail in Chapter 2. It was shown by repeated control testing between the two observers that practically identical results were obtained without the one knowing of the results of the other. With regard to the differential count of the white blood cells, only those cases which were examined by the one or the other of the above-mentioned observers are included. For practical reasons the entire material of patients could not be examined in this manner, which is the reason why the number of cases with recorded results from the differential count was reduced to 160.

In order to study the contagiousness in the material, attempts were made in 1956 to examine contacts to the patients. 57 such contacts have been examined clinically and haematologically. 19 of these had to be withdrawn from the results, however, since it was found either that one and the same patient had more than one contact or, in a few instances, that the contact referred to more than one patient. In the analysis, the contact material was reduced by these 19 instances and amounted, according to criteria stipulated in Chapter 6, to 38.

Finally the occurrence of atypical mononuclear cells was investigated in healthy individuals. This material consisted of three age groups of children from the Engelbrekt Elementary School and stu-

dent adults from the Royal Central Gymnastic Institute in Stockholm. With the intention of obtaining a material of healthy patients as free from infection as possible, this examination was organized during the spring of 1958 so that it could be carried out during the first week of the coming autumn term, i.e. on the return of the children and students to their respective teaching establishments immediately after the summer vacations. This was done according to plan and the results are given account of in Chapter 7. This material consisted of 82 children in the age group 7—9 years, 138 in the group 10—12 years, 60 in the group 13—16 years and 37 adults.

The differential count of the white blood cells from the contacts and the healthy material was carried out in the same special manner as described for the patients, i.e. only by the one or the other of the above-mentioned observers.

The haematological result was analyzed statistically with special regard being paid to the quantitative occurrence of the atypical cells. In this context the main problem was to what extent a significant difference was present between 1) various groups of the patient material (children/adults, males/females or sero-positive/sero-negative cases), 2) patients and contacts and 3) contacts and healthy individuals.

Historical Background

General Review of the Literature

Infectious mononucleosis is looked upon today as being identical with idiopathic ademtis, Drüsenfieber" glandular fever and monocytic angina.

It is characterized by hyperplasia of lymphoid tissue fever the occurrence of atypical mononuclear cell elements in the peripheral blood and at times in various organs within the organism—factors de termining the classification of the disease as systemic. In certain cases it has the ability to produce a heterophilic agglu-tinin to the blood corpuscles of sheep The disease is found all over the world and no clear seasonal variation can be said to occur

Modern writers on the disease usually divide its history into three phases a clinical, a haematological and a serol ogical. In so doing, however a number of previous reports on blood findings are not taken account of and thus the divi-sion into a clinical and haematological phase is not strictly speaking correct.

The following historical account of the disease is of a summary nature and those interested in details are referred to more extensive previous papers on the subject (28, 84 114 115 117 128, 136, 137 145 197 198, 221)

Early Papers In 1885 Filatov professor of paediatrics in Moscow reported his observations on juvenile idi ... vical lymp ... e R of his lect ... o in ch ... greater international spread by his Ger man paper entitled "Semiotik und Diag nostik der Kinderkrankheiten (71) and by the German translation of his lectures in 1897 (7?)

At a scientific meeting in Cologne in 1889 Pfeiffer (146) described "Drüsenfie ber" a diseased state characterized by swelling of the cervical glands, enlarge-ment of the liver and spleen, and fever—a stat which was of epidemic occurrence and for which the prognosis was favoura ble. At the same meeting Heubner (95) described several cases of his own one of which exhibited exanthema. Drüsen fieber" has long been the dominating term in German medical literature being used for instance in the major works on the subject by Glanzmann (82, 83 84) and Lehndorff-Schwarz (114 115)

The disease was first described in the United States by West in 1896 (217) and in Britain by Williams in 1897 (220) Also in 1897 Cantlie (39) reported that n years previously in Hong Kong he had seen children suffering from a non-malig nant swelling of the glands of the neck, subsequent to fever without sore throat. In 1905 Korsakoff (110 111) described the possibility of complication in the form of acute glomerulonephritis. In 1907 Türk (?06) reported on a case having clinical signs which are nowadays recognized as those of infectious mononucleosis. This uthor observed a total white cell count of 16,700 and a differential lymphocyt count of 62.7 % of which, as he himself says, *...ber were enlarged and atypi* ... considered the diagnosis to

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Laboratory Methods:

Haemoglobin was determined as oxy-haemoglobin.
Thymol was determined according to Mac Lagan.
Bilirubin was determined according to Jendrassik-Grof.
Prothrombin was determined according to Quick.
Thrombocytes were determined in capillary blood according to Kristenson.
Reticulocytes are calculated per 1,000 erythrocytes on smears stained with brilliant cresyl blue.
Serum iron was determined according to Agner.
Protein in urine was determined with sulfosalicylic acid.

The peripheral blood smears were stained, prior to the differential lculation, in the following manner.

The smear was suffused with May-Grünwald's stain for 30 seconds and then washed in distilled water. Remaining water w shaken off by hand. The smear was then suffused with the Giemsa stain for 20 minutes. This was also washed off with distilled water after which the smear was placed on its side to dry in the air.

May-Grünwald's stain

30 g eosin-methylene blue according to May-Grünwald per litres of methyl alcohol.

Giemsa's stain

60 g azur 2 eosin Merck
16 g azur 2 Merck
5 kg glycerin
Methanol *ad* 10 litres

In this work the term Total Leukocytes refers to the various white blood corpuscles taken *as a whole*.

The term R & S Leukocytes refers to the rod-nucleated and segmented leukocytes belonging to the myeloic series.

CHAPTER I

Historical Background

General Review of the Literature

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It is characterized by hyperplasia of lymphoid tissue fever the occurrence of atypical mononuclear cell elements in the peripheral blood and at times in various organs within the organism—factors determining the classification of the disease as systemic. In certain cases it has the ability to produce a heterophilic agglutinin to the blood corpuscles of sheep. The disease is found all over the world and no clear seasonal variation can be said to occur

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his part sets the following criteria for diagnosis 1) positive heterophile reaction, 2) that the lymphocytes at some stage during the course of the disease account for more than 50 % of the leukocytes and 3) a characteristic picture of clinical symptoms.

Mason & Adams (126) analyze 100 cases and consider that it is possible to base *definitive* diagnosis on 1) clinical findings *and* 2) a characteristic blood picture (which the authors calculate as being 50 % or more lymphocytes and 15 or more atypical cells of the total white blood cells) *and* 3) a positive heterophile test and a *presumptive* diagnosis of 1) compatible clinical findings *and* 2) a characteristic blood picture *or* 3) a positive heterophile test. Leibowitz (118) who earlier had required seropositivity for the diagnosis of infectious mononucleosis, agrees in this article from 1956 that sero-negative cases may occur and that the diagnosis is to be considered reasonable if 50 % of atypical cells are to be found in such cases.

Serology

In 1911 Forssman (76) described an antigen-antibody reaction in which it was found possible by the injection of organ emulsions from guinea pigs into rabbits to stimulate the production of haemolysin with specific effect on the blood of sheep i e a heterophile antibody This discovery has been of great importance in the field of serology and the term Forssman antigen stems from it.

In 1932 Paul & Bunnell (141) demonstrated heterophile antibodies in patients with infectious mononucleosis. In spite of similarities regarding basic theories (117) it can be shown by absorption tests that the heterophile antibodies in patients with infectious mononucleosis are not of true Forssman nature (28 50, 52) Absorption tests are now considered of the greatest importance for assuring the specificity of the reaction and various methods have been described, for example (14 49 116, 167) The Paul Bunnell reaction complemented by absorption tests with guinea-pig kidney extract and ox erythrocytes is now considered to be of great support for the diagnosis (28 117 211) However in a few conditions (rheumatoid arthritis, Hodgkin's disease tularaemia and diabetes) false sero-positive tests have been seen (51)

A screening test for infectious mononucleosis has been described (193)

The importance of the Paul-Bunnell reaction was emphasized early on (10 165) However a difference of opinion arose among clinicians as to whether seropositivity should be set as a criterion for diagnosis or not. Many consider that a positive Paul Bunnell reaction should not be demanded, even though the sero-positive cases constitute a more solidly diagnosed group (62, 66 81 98, 118, 131 148 184 186 197 198) Other authors hold fast in their demand for a positive reaction (18 51 96, 97 107 120 177) Mason & Adams (126) differentiate between definitive diagnosis in which a heterophile test is included and presumptive diagnosis in which such may be absent.

It has been pointed out that there is a greater chance of a positive reaction in children over 14 years of age than in younger children (19 227) Vahlquist, Ekelund & Tveterås (211) found no positive heterophile test in children under the age of 5 Belfrage (17) on the other hand, reports 6 children under the age of 5 to have had a positive Paul Bunnell reaction (even though he judges the reaction positive first at a titre of 80 Vahlquist *et al* at 40 and Bennike (19) at 32)

The reaction can be transient (199 211) it can appear early (18) or late (6, 199 211) in the course of the disease. Bennike (19) stresses the value of repeated tests, in particular during the first month, and believes that fluctuation between a positive and a negative Paul-Bunnell reaction occurs more often in children than in adults.

Bailey & Raffel (5) demonstrated the occurrence of ox cell haemolysin as well as sheep cell agglutinin in the sera of patients suffering from infectious mononucleosis. There is, however no absolute parallelism between the occurrence of sheep agglutinin and ox cell haemolysin in such patients. It seems as if the presence of ox cell haemolysin is a more delicate test than the Paul-Bunnell reaction (62, 63 143)

Gleeson, White, Heard, Mynors & Coombs (85) have shown an excellent ox cell agglutinability in infectious mononucleosis sera provided that the surface of the cells is digested for a short while beforehand with trypsin. On the basis of this Tomcsik (202) elaborated a simple test for clinical use in which absorption with a suspension of guinea pig kidney precedes agglutination and gives it a very high degree of specificity

Pathological Anatomy and Reports on Biochemical Disturbances

In 1927 Halen (89) reported on three cases in which death had occurred and post mortem examination had been carried out. The truth of the diagnosis is to be questioned, however because of bacterial findings in the throat—diphtheria bacilli in two cases and staphylococci in the third.

In 1947 Allen & Kellner (1) were able to perform a post mortem examination of a patient who had died in an air crash and found focal cellular infiltrations in the liver kidneys, heart, lungs, suprarenals, testes and brain. The infiltration consisted of atypical cells and small lymphocytes. Custer & Smith (47) present a pathological-anatomical survey of previous post mortem cases and contribute with 9 of their own. They observed infiltrative changes in the lymph glands, spleen, respiratory organs, tonsils, lungs, cardiovascular organs, peripheral vessels, stomach, ileum, liver pancreas, kidneys, bladder prostate testes, suprarenals, thymus, hypophysis, nervous system, musculature and skin (corium)

In the early days the morphology of the bone marrow was reported as being normal (134) but since then certain cases have been found to exhibit granulomatous changes on sternal puncture (101 14)

Rupture of the spleen is one of the more dramatic manifestations of the disease and has been described a number of times (33 74 178, 182, 191 213, 226) Ziegler (226) reports that the spleen in his cases exhibited intensive diffuse infiltration with mononuclear cells and proliferation of the reticulum.

Thrombocytopenic purpura (48 87 223) and prothrombin deficiency (191) have been observed.

Jersild (104) describes a case of death from infectious mononucleosis which was complicated by myocarditis. This was a 25-year-old man with sero-positive infectious mononucleosis who died 8 days after onset of the disease

Cases of icterus have been described (107 222) Nelson & Darragh (131) studied the functioning of the liver in 22 young men with clinically diagnosed infectious mononucleosis and were able to demonstrate liver involvement in them all. Involvement of the reticulo-endo-

thelial system has also been described in instances of liver biopsy (15 163) Bang & Wanscher (11) report on 4 cases of icterus on which liver biopsy had been performed.

They found parenchymatous and inter stitial inflammatory changes and prolif eration in the sinusoids of small lymphocyte-like cells presumably belonging to the reticulo-endothelial system. Sullivan, *et al* (189) made a comparison of results of liver biopsy in infectious mononucleosis patients and patients with viral hepatitis. The infectious mononucleosis patients exhibited inflammation cells in the hepat ic sinusoids and in the portal region but no parenchymal damage From this the authors believe that it is possible to differ entiate the diseases *histologically* one exception, however being the most seri ous cases of infectious mononucleosis in which involvement of the parenchyma was observed. Reichman, Burke & Davis (156) describe a case of infectious mononucleosis with liver damage studied by serial liver biopsy in which they found lesions indistinguishable from viral hepa titis Leibowitz & Brody (119) describe a case of cirrhosis of the liver after infectious mononucleosis, diagnosed by liver biopsy In 1960 Bennike (20) published the monograph Studies on the Involvement of the Liver in Infectious Mononucleosis from the Blegdam Hospital in Copenhagen. His main emphasis is laid on cell infiltration, opposed to which parenchymal lesion is afforded far less importance even though certain focal damage may occur in the most exposed areas He considers that cirrhosis in infectious mononucleosis is very uncommon and most often probably occurs in combination with other features of damage.

Changes in the serum lipoproteins 166 and serum glutamic puruvic transaminase (112) are reported on. Rosalki, Jones & Verney (164) describe increases in serum glutamic-oxalacetic transaminase and glutamic puruvic transaminase. The increases appeared during the first week of illness, were usually maximal during the second week and most often returned to normal within five weeks. As a rule conventional liver tests were less often abnormal.

Belfrage (16) states that in the acute stage it is usual to find normal fibrinogen but slightly elevated $alpha_2$ globulin in the plasma. The increase in gamma globulin may remain as an isolated disturbance for a longer or shorter period.

Involvement of the Central Nervous System

Involvement of the central nervous system in infectious mononucleosis has been afforded increasing attention during re cent years (29 198, 199) Involvement of this kind has been in the form of meningitis (105 215) encephalitis (70 115 133 181 197 212) meningoencephalitis (61 79 121 154) peripheral paresis (160, 168) polyradiculitis (44 109 152, 158, 161 186) optical neuritis (70 106) and meningo-encephalo-myelitis (54)

The diagnosis of such cases was often decided on with the aid of a lumbar puncture which indicated a certain increase in pressure and usually a moderate increase in the number of cells. As a rule this pleocytosis was found to be largely due to an increase in the number of mononuclear cells. A moderate increase in albumin was often found (197) At times it was possible to demonstrate atypical cells of the same type as those found in the peripheral blood (99) and at times it was found possible to obtain a

positive Paul Bunnell reaction in the cerebro-spinal fluid *(ibid.)* At other times EEG examination was of assistance (113, 157) Examination of the cerebro-spinal fluid and EEG indicates a higher frequency of irritation of the central nervous system than would appear from current methods of clinical examination (212) The value of repeated EEGs is emphasized (21)

The first to describe such involvements of the central nervous system was Hecht Johansen in 1931 (105) when he reported on a case of serous meningitis in a 28-year-old man suffering from infectious mononucleosis. In this instance all symptoms disappeared within three weeks. That same year Epstein & Dameshek (61) described a similar but more acute case in which there was also complete recovery Among the more lengthy writings on the subject of infectious mononucleosis which are worthy of note is the thesis from 1942 by Thomsen (197) in which the material consists of 549 cases. Of these, 27 showed clinical signs of involvement of the central nervous system, this being 4.9 % The cerebro-spinal fluid of 21 of these 27 cases was examined 16 of these 21 showed pleocytosis. Thomsen also examined the cerebro-spinal fluid of 33 patients who did not show symptoms of involvement of the central nervous system. 21 of these 33 were found to have pleocytosis He performed the Paul-Bunnell test in 3 cases, all of which proved to be negative. There were 7 instances of death in the entire material, the mortality thus being somewhat greater than 1 %. 5 of the fatal cases showed no other complication to the original ailment of infectious mononucleosis and were due to central respiratory paresis. Two of these cases were subjected to post mortem examination and were found to have fresh degenerative changes in cells belonging to the respiratory centre.

The Occurrence of the Atypical Cells in Various States

The atypical cells did not occur in infectious mononucleosis alone (32, 57 86, 96 100, 103 117 118, 183, 185 188, 222) Meythaler & Häupler (128) state that it must be established that a special blood picture for infectious mononucleosis is neither to be found nor to be expected. The blood picture of this disease with its lymphocellular and plasmocellular reaction, is, they say merely an expression for a state of irritation in the basic reticular tissue in the lymphatic organs. However an organic system reacts in the same way to different stimuli. In analogy to this, similar blood changes are found in other diseases with affinity to lymphatic tissue, especially in virus diseases. So early a paper as that by Haberfeld & Aster-Haberfeld (88) from Brazil in 1914 mentions pathological forms of lymphocytes with a description which in many respects agrees with Downey' (56) in patients with enlarged lymph glands after tick bites. Litwins & Leibowitz (122) prefer to regard the cells as virocytes since they are considered to occur in several diseases of virogenic origin. However they are to be found in other than virogenic diseases, i.e in a number of bacterial diseases (7 23, 24 25) in malaria (3?) and also in allergic states (177) In an experimental study on animals, Dougherty & Frank (55) found the atypical cells in states of stress in which involution of the lymphatic organs also occurs often (174) With regard to this wide range of occurrence Fichtelius & Wahlquist (69) choose to designate the cell *atypical* and all the more so since the questionable morpho-

logical differences between them are so subtle that it is not possible to differentiate them in the general clinic. Finally it may be mentioned that various descriptions of the atypical cells in healthy individuals have also been given (32, 69 155)

Aetiology and Infectiosity

The aetiological agents of the disease are still unknown.

Nyfeldt describes the disease (136) and considers himself able to show that its cause is due to *B. monocytogenes hominis* a bacterium which he himself demonstrated (137) and was later able to refer to the Listerella (138) Wising (221) did not succeed in reproducing Nyfeldt's work on *B. monocytogenes hominis* although he was able to transfer clinical symptoms of the disease to monkeys by the intra cerebral, intraperitoneal or subcutaneous injection of a fresh suspension of lymph glands from patients. Signs of the disease appeared in one instance in man after the transfusion of 250 ml of heparinized blood from patients. Lymph glands were examined microscopically in 16 cases, showing changes which were considered explainable as being caused by a virus. The author concludes "The results of these investigations would seem to provide support for the assumption, previously based on clinical grounds, that human infectious mononucleosis is due to a virus with a specific lymphotropic affinity"

Bang (8, 9) contributes to the discussion on the aetiology of the disease and shows that the susceptibility is slight and indicates that Wising's investigations speak greatly in favour of the virus theory especially in view of the numerous negative bacteriological findings. The current view is indeed that the disease is caused by a virus (17 63 64 66 163 218, 221 224) The low infectiosity is emphasized from many quarters (8, 9 17 63 65 84 96, 98, 132, 199) However according to Petrides (145) and, if the term "pseudo-mononucleosis" introduced by Vahlquist (210) is included, according to Vahlquist Ekelund & Tveteras (211) it seems to be clear that even if infectious mononucleosis as a rule has an underdeveloped infectiosity as regards a more complete clinical picture its genetic agent has a comparatively large capacity of spread (86) and the capacity of developing haematological changes without subjective or other objectively demonstrable symptoms (90 98, 127 214)

Discussion of Selected Papers Dealing with Problems of Differential Diagnosis

Pfeiffer (146) made the early statement "Ohne Zweifel wird dieses klinische Bild, wie so viele unserer klinischen Krankheitsbilder verschiedenartige Krankheitsprozesse in sich begreifen, und erst bakteriologische und patologisch-anatomische Forschung wird dann im Stande sein, die einzelnen ätiologisch und anatomisch verschiedenen Formen zu sondern

Korsakoff (111) also maintained at an early stage that it was in fact impossible to know whether this is a disease *sui generis* or whether it is a reaction or rather sub-reaction to one or more agents.

The aetiology of the disease is still unknown.

Nyfeldt (137 138) considered *Listerella* to be the cause of the disease That a demonstration of this has not been able to be reproduced does not mean so much from today's viewpoint. There is nothing to show that Nyfeldt's cases were not in fact Listerelloses in which an atypicality attended the blood picture as in many other bacterial diseases. Moreover Reyer

bach & Lenert (159) induced a monocytic reaction in rabbits by the injection of *Lister lla monocytogenes*

Goldthwait & Eliot (86) maintain that one should not be dogmatic in setting the diagnostic criteria for infectious mononucleosis since the aetiological agent is unknown and the agent/host relationship unelucidated.

Against this background it is understandable that a number of very difficult problems of differential diagnosis are at hand. Belonging to these are *atypical mononucleosis* (4) and *pseudomononucleosis* (69 210 211) the latter being nearest equivalent to infectious mononucleosis without tonsillitis and with a negative Paul Bunnell reaction but in which several other clinical data are otherwise identical with those of infectious mononucleosis.

Vahlquist, Ekelund & Tveterås (211) state how seldom a positive Paul-Bunnell reaction is found in infectious mononucleosis in children under 5 years of age. On the other hand the literature on the subject contains examples of sero-positive cases of the disease in children, according to Belfrage (17) down to 3 years of age and to Phillips & Stone (147) down to an age of 6 weeks. If one refers to the literature previous to 1932 when Paul and Bunnell published their serological findings it is possible to find a few examples of cases under 5 years of age (129) and down to an age of 7 months (53, 149)

Shubert, Collee & Smith (177) describe 27 cases of what they chose to call an epidemic variant of infectious mononucleosis. These cases were sero-negative and the number of relative lymphocytes was often less than 50 %

Evans (63) proposes a hypothetical explanation for the various clinical forms of infectious mononucleosis and is referred to by Wickström (219) Evans suggests arrangement of the disease into 3 phases

1) Mostly seen in children who have fever and respiratory symptoms. This is the epidemic variant described by Shubert *et al* and it has a short incubation period. This stage is often passed by unnoticed. At times it develops into 2)

2) This is where the viraemia makes its appearance. The patient has fever adenopathy and often a rash. Lymphocytosis is apparent but to no very great degree Heterophile antibodies may be present in low titre but may also be absent. Phases 1) and 2) are probably the juvenile forms of the disease.

3) Represents the consequences of the tissue reaction. Lymphocytosis is present here with a high frequency of atypical cells. If the reaction is powerful the case is sero-positive. This phase is typical for young adults. The earlier phases may have passed by unnoticed, explaining the reports of a long incubation period. Evans believes that the infectiosity is stated to be low because individuals pass unnoticed through phase 1) and 2) and in this way attain immunity

Infectious lymphocytosis as described (179) by C. H. Smith in the American Journal of Diseases of Children and later supplemented in J.A.M.A. (180) consists of cases which on careful reading appear to have cells with atypicity The degree of difficulty in drawing up a demarcating line here is well illustrated by the fact that Vahlquist (211) is inclined to refer the latter phase of some cases of pseudomononucleosis to infectious lymphocytosis. Hoagland (97) considers that infectious mononucleosis is rare in the lower juvenile age groups and that such illness in the majority of children is due to an

acute fever which is clinically similar to infectious mononucleosis. Such a state usually gives a negative Paul-Bunnel reaction. Haematologically this disease generally distinguishes itself from true infectious mononucleosis. Berg (22) reports on several cases of children as well as adults with infectious lymphocytosis in which the occurrence of atypicity is denied. It should therefore be possible to exclude Berg's cases from the discussion on differential diagnosis as an independent group, that is to say as *infectious lymphocytosis* but on the other hand it should be remembered that C. H. Smith's cases are clearly more closely related to infectious mononucleosis. It is possible that Berg's cases are more in affinity to Hoagland's description, even though Berg's material comprises adults as well as children.

It may also be discussed whether there is one mononucleosis virus or several. A support for the belief that there are several might be the so very different reports on the incubation period. Thus Petrides (145) says 1—9 days, Ollgaard ( ) 4 days, Wechsler Rosenblum & Sill 15) 7—9 days (generally 9 days) T d 199) 5—11 days (7—10 days in the majority of cases) Davis (53) 10 day weeks, Harnes & Mitman (92) 5 da 2 weeks (exceptionally 3 weeks) Glanz nn (84) 5 days—2 or 3 weeks, Evans 64) 7 days—2 weeks, Rominger (163) 11 days—2 weeks (at times 3 weeks or longer) and Hoagland (97) 33—49 days. It is possible that there are one or more viruses which give a quite typical clinical picture in combination with a positive Paul Bunnell reaction, and another or others which give a similar picture but lack a positive Paul-Bunnell reaction. Thus Belfrage (17) reports on a large material and on the basis of clinical symptoms believes that the sero-positive and sero-negative juvenile cases in it have the same genesis whereas in a higher age group (>23 years) sero-positive and sero-negative cases are caused by different agents. Whitby & Britton (218) for their part consider that several closely related viruses may be the cause of the complex of symptoms and that of these the majority give a positive Paul-Bunnell reaction and the remainder none. A further support for the theory that there might be several mononucleosis viruses are the rare reports of recurrent infectious mononucleosis (75 140) Of the greatest interest is the state in infectious hepatitis (cf the particulars on liver damage in the section on pathological studies) in which atypical cells are commonly found in the peripheral blood and to a particularly high degree in cases with palpable cervical glands (93) Differentiation between infectious hepatitis and mononucleosis can in this respect be most difficult (13 26, 43 93 131 156 189) Strom (187) however considers that palpable cervical lymphomata are often absent in infectious hepatitis even though the occurrence of atypical cells is clear

CHAPTER 2

Cytology

All of the three forms described by Downey (56) have been found in the present material. Downey & McKinlay's paper (56) gives an extensive and very good description of the cells with excellent colour illustrations. The reader interested in this aspect is primarily referred to this work. All that will be given here is a summarized review of the cytology.

Type I consists of a group of cells which may vary a great deal both in size and form. As a rule these cells are larger than the normal lymphocyte and may even be larger than a normal monocyte.

The nucleus is of varying size and may be round as in the normal lymphocyte. However it is more often irregular horseshoe-shaped and quite often deformed. The internal structure is always lymphatic, i.e. the chromatin forms a network of broad bars which are not sharply defined from the parachromatin, thus giving a blurred appearance. In certain nuclei it is possible to see a denser form of chromatin with a tendency to separate from the parachromatin, resulting in a clearer picture. In certain larger cells the chromatin may be of a less dense character but never to such a degree that the cell might be mistaken for a blast cell, for example. A few of the cells in Downey's Type I contain nucleoles, but they are surrounded by large blocks of chromatin so that the appearance of the nucleus is nevertheless of lymphocytic character.

Thus even though the lymphocytic nature of the nucleus is always obvious, the cytoplasm deviates just as clearly from that of the normal lymphocyte. The degree of basophilia varies somewhat, but the majority of cells are very basophile, much more so than is usual in normal lymphocytes. Many of the cells show a basophilia typical of a plasma cell, but not, on the other hand, of the nucleus of the plasma cell. Characteristic is that the cytoplasm has a frothy appearance and quite often possesses vacuoles, this being due to the uneven distribution of spongioplasm. In the periphery the cytoplasm is often darker and more homogeneous because of larger amounts of spongioplasm. In the vicinity of the nucleus, on the other hand, there is a greater amount of hyaloplasm, making this zone of a lighter shade. Many of the cells contain azurophile granules, the larger cells in particular sometimes exhibiting great numbers of them.

Type II. The nuclei of this type generally have the same form as that of a large lymphocyte, its internal structure likewise. The deviation from the normal in a number of instances is a resemblance of the nucleus to that of the plasma cell. The bars of chromatin are thick, and dense rounded or angular blocks of chromatin often appear. This, however, is here not so markedly spoke-shaped as in the nucleus of the plasma cell. The nucleus is very delicate and may easily be damaged.

The cytoplasm differs from that in Type I primarily by less basophilia. It has fewer vacuoles. Azurophile granules may be present. The spongioplasm is of a more even appearance which does not

give the frothy porous effect seen in Type I. Characteristic of many cells in this group are broad basophile bands running radially from the nucleus in a relatively large cytoplasm. On rare occasions, however, the basophile area may be without the radial arrangement.

In summary it may be said both of Type I and Type II that they are clearly modified lymphocytes whose deviating character is believed to be dependent on a greater degree of maturity and on a functional activity in these cells (56).

Type III is met with in few patients. This cell type resembles the lymphoblast in structure and in size may be even larger than a lymphoblast. The nucleus is leptochromatic with certain haziness in the delineation. Nucleoli are present occasionally (cf. Türk's "Reizungsformen") (205). The cytoplasm is often highly basophile with the basophilia increasing towards the periphery. Here as in Type I there is commonly a light, perinuclear zone. The cytoplasm nearly always has a frothy appearance and very often a tendency towards vacuolation, at times large vacuoles. Now and again one large vacuole may contain one or more azurophile rods, Auer's azure rods (56, 115). Azurophile granules are present in the cytoplasm to a moderate extent.

With regard to the chromatin structure, it is probably correct to look on Type III as a very young cell which is closely related to the immature cell, whereas Type I with its marked delineation of the chromatin and haziness at the boundary between chromatin and parachromatin is undoubtedly an older cell (56, 139).

Intermediate forms between lymphocytoid and monocytoid cells occur in the material similarly to the materials of Glanzmann (84) and Lehndorff & Schwarz (115). Here we have a mono-

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Fig. 1. Colour Plates of Atypical Cells.

a. 1260. Example of Downey's Type I. Not particularly large compared with adjacent erythrocytes! The nucleus relatively large, suggestion of kidney shape. The heavy chromatin in large bars or clumps. The cytoplasm generally highly basophilic and foamy.

b. 1..0. Another example of Downey's Type I. The cell large. The nucleus only slightly eccentrically situated, somewhat irregular outer contour. The chromatin heavy with indefinite demarcation, the nucleus bars. The cytoplasm only moderately basophilic, clear foaminess and certain occurrence of azurophile granulae.

c. 600 Downey Type II. The cell large. The nucleus round, somewhat damaged. The bars of chromatin thick, tend to be rounded. The cytoplasm fairly light with greater basophilic concentration in the lower left-hand region, otherwise even appearance with only few vacuoles and some azurophile granulae.

d. 800 Downey Type III. The cell fairly large, polygonal in form. The nucleus almost horseshoe-shaped, leptochromatic, the chromatin well separated from the parachromatin. The cytoplasm highly basophilic, increasing basophilia in several places towards the periphery. Marked foaminess.

e. 1..60 Downey Type III. The cell large, rounded. The nucleus likewise rounded, only slightly eccentrically situated, leptochromatic, somewhat hazy in appearance but exhibiting one or two nucleoli. The basophilia in the cytoplasm ordinary. Little foaminess in this instance. Moderate amount of azurophile granulae.

f. 1 N0. Large cell of [illegible] character [illegible] ... trically situated

having an irregular outer contour. The [illegible] ... phile granulae

[illegible] cytoplasm. I[n] the lower right-hand [illegible] ... almost hya-

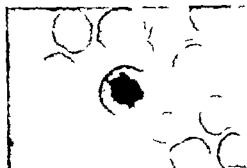
[illegible] I[n] the upper part of the central area large [illegible] ... oplasm.

a



b





d





f



philes in movement, not at rest. According to Senda the tail consists of gelatinized cytoplasm which is coherent and elastic.

I have been unable to find any reference in the literature to studies of this type on the atypical cells in question here (Downey's or closely related types)

Concerning the vacuoles in the cytoplasm, which are so very characteristic of the atypical cells, it may be said that several studies employing methods of phase contrast or interference have been carried out in attempts to show their genesis in blood cells (31 91 196) Various explanations have been put forward, but no definite scheme for the mode of formation of the vacuoles can be found (91 196)

In the literature the atypical cells are as a rule referred to the lymphatic type (56 58, 82 94 114 130, 134 170 171 173 185) Nordenson (134) maintains with some emphasis that the pachychromatic structure of the nucleus and the negative oxidase reaction speak in favour of this. Later however he reports that the oxidase reaction varies and can be positive (135) Forms intermediate to monocytes which are difficult to evaluate are found (82 94) and differentiation between the lymphocytoid-monocytoid-plasmocytoid cell may be impossible at times. It is not improbable that this is a question of a very lively proliferation which brings to mind the theory according to Mayer (73) concerning the ability of the tissue cells with regard to dedifferentiation and pluripotentiality

The problem becomes somewhat simpler if one follows the so-called *biological leukocyte curve* according to Schilling (169) with 1) neutrophile attack phase 2) monocytic defence phase and 3) lymphocytic-eosinophile recovery phase in which room is afforded both monocytoid and lymphocytoid cells. Beyreder & Herzog (32) however refuse to see any other cells than lymphocytoid, referring in support of this to the theory put forward by Kluma the head of their clinic in Vienna, concerning the reactibility of the lymphatic system as opposed to the reaction of the myeloic system. It is their opinion that the lymphatic reaction is chronologically the same as Schilling's attack phase.

Of easier verification is when it is suggested that the lymphatic reaction is by no means always accompanied by an increase in the number of lymphatic cells (32 130) that it is often merely a question of qualitative changes in which the lymphatic cells may be reduced in absolute number Even so there is often enlargement of the lymphatic organs with a palpable spleen and lymph glands in such cases.

Of far greater interest, however are the discussions of recent years on the lymphocyte and its ability with regard to pluripotentiality (40 225) Here we have a return to the features of Mayer's (73) theory from 1931 Similarly it is also maintained that the white blood cells are formed from a common reticular cell and that various possibilities of development are present (155 190) It is suggested that a "conditioning" of the lymphocyte may take place by the exposure of the individual to antigens (225) but information as to whether this "conditioning" is marked by special morphological features is lacking

CHAPTER 3

Clinical Studies

a. Main Study

As will have appeared from the indroductory historical survey infectious mononucleosis exhibits a series of clinical manifestations. Table 1 and 2 presents information on the frequency of various symptoms in the material of patients on which the cytological studies presented in this work are based. The material consists of the cases of infectious mononucleosis diagnosed at the Hospital for Infectious Diseases in Stockholm during the years 1955 and 1956. Such diagnosis at the hospital is made clinically and not serologically

Infectious mononucleosis is a state of disease which is characterized by relatively protracted, fluctuating pyrexia, almost always enlargement of the lymph glands in one or several regions, relative lymphocytosis and the appearance in the blood of atypical cells without signs of malignancy Tonsillitis is also usually present, in roughly every other case, splenomegaly and, to a somewhat lesser extent, hepatomegaly Heterophilic antibodies appeared in the blood in somewhat more than half of the patients.

The first section in table 2 comprises the entire series of patients and is referred to as the total material. The second section comprises cases investigated in the haematological study referred to in the Introduction. These are identical with the cases included in Chapter 4 and 5 this section is referred to as the special material. The third section, finally consists of the special material divided into sero-positive and sero-negative cases, into males and females and into adults and children, the children in this instance comprising cases up to and including the age of 14. Where the designations cases without secondary infection" and cases with secondary infection occur the basis for division in parts a and b of this chapter is the same as in chapter 4 b, i.e. the group with secondary infection comprises patients who apart from infectious mononucleosis have displayed symptoms of another infectious disease, most often bacterial in origin.

Each column contains a number followed by the percentage constituted by this number of the whole group in question.

The recordings are of tests performed during hospitalization or during follow-up in the out-patient clinic of the hospital. The number of samples taken from each individual varied, and so the table cannot be used as a basis for statistical analysis of the tests.

Erythrocyte Sedimentation Rate (ESR) Maximal ESR was recorded. A moderately increased ESR of 12—30 mm/hour was found to predominate. The frequency of higher ESRs (> 30 mm/hour) was moderately great, however

When dividing the total material into age and sex groups, this tendency was noticeable in all groups except that of adult men which showed the same frequency of normal as greatly increased ESRs (22.1 % each)

The special material showed an insignificantly greater frequency of increased ESRs compared with the total material. Among the sub-divisions of the special

TABLE 1 *Patient material of infectious mononucleosis during t e year 1955 and 1956 (the total material)*

	Males	Females
0–4 years.	6 = (86%)	1 = (14%)
5–14 years.	46 = 5_3%	42 = 47 7%
Adults	77 = 53.8%	66 = 46.2%
Total	129 = 54.2%	109 = 45.8%

material there was a higher frequency of normal ESRs in the sero-negative than in the other groups.

Haemoglobin Minimum Hb values were recorded, the lower normal limit being drawn at 11 g %

When dividing the total material in this w) 1/3 of the children were found to exhibit anaemia (as rule initially)

The special material displayed a some what greater frequency of anaemia than the total material. On dividing the spe cial material into sub-groups it was found that sero-positive cases were anaemic more frequently than sero-negative females more frequently than males and hild en much more frequently than dults

P) a This wa counted as being a empe ature of 37 6 C or more. The durations recorded indicate the length of the period of pyrexia in question from reception at the hospital up to and in cluding the day after which the tempera ture of the patient no longer reached 37 6 C Pyrexia was pparent in the vast majority of patients on entering the hospital

A pyretic period f up to two weeks dominated.

On sub-division of the otal material it was found that children ended t have shorter py etic period than dults

The total material and special material exhibited relatively close agreement.

On division of the special material it was found that there was a tendency towards a shorter pyretic period among sero-negative cases than among sero-positive that females had a greater spread of pyretic duration than males and that children, as in the total material, tended to have a shorter pyretic period than adults.

Eyelid and facial oedema This symptom appeared in a little less than 1/5 of cases, both as regards the total material and the special material.

On division of the special material it was found that there was a greater fre quency among sero-positive cases than among sero-negative and a similarly greater frequency among females than among males.

Sinusitis The diagnosis was made by X-ray examination.

In the total material, children displayed a greater frequency of positive results than the adults.

The special material showed a some what greater frequency of sinuitis than the total material.

On division of the special material it was found that there was a greater fre quency of pathological findings among sero-positive cases than among sero-negative among males than among females and among children than among adults. Statistical analysis according to the χ^2 method did not, however prove the differences to be significant.

Palpabl spleen The spleen was found to be much more often palpable than the liver

and the the into d to be

TABLE 2. *Various clinical symptoms in the total material and the special material with further division of the latter into sero-positive and sero-negative cases, males and females, and children and adults*

	ESR			Hb	
	Normal	12—30 mm	> 30 mm	Normal	<11 g%
Total material	32—13.4%	119—50.0%	87—36.6%	195—81.9%	43—18.1%
Special material	14— 8.8%	82—51.3%	64—40.0%	122—76.3%	38—23.8%
The special material divided into					
) Sero-positive cases	7— 6.3%	59—52.7%	46—41.1%	83—74.1%	29—25.9%
Sero-negative cases	7—14.6%	23—47.9%	18—37.5%	39—81.3%	9—18.8%
b) Males	8— 9.9%	42—51.9%	31—38.3%	65—80.2%	16—19.8%
Females	6— 7.6%	40—50.6%	33—41.8%	57—72.2%	22—27.8%
) Children	5— 7.4%	35—51.5%	28—41.2%	40—58.8%	28—41.2%
Adults	9— 9.8%	47—51.1%	36—39.1%	82—89.1%	10—10.9%

	Pyrexia				
	0	1 day— <1 week	1— <2 weeks	2— <3 weeks	3 weeks—
Total material	1—0.4%	86—36.1%	96—40.3%	39—16.4%	16— 6.7%
Special material		51—31.9%	70—43.8%	25—15.6%	14— 8.8%
The special material divided into					
) Sero-positive cases		33—29.5%	51—45.5%	19—17.0%	9— 8.0%
Sero-negative cases		18—37.5%	19—39.6%	6—12.5%	5—10.4%
b) Males		23—28.4%	40—49.4%	14—17.3%	4— 4.9%
Females		28—35.4%	30—38.0%	11—13.9%	10—12.7%
) Children		33—48.5%	23—33.8%	7—10.3%	5— 7.4%
Adults		18—19.6%	47—51.1%	18—19.6%	9— 9.8%

	Eyelid and facial oedema		Sinusitis		Spleen	
	Absent	Present	Absent	Present	Not palpable	Palpable
Total material	193—81.1%	45—18.9%	95—62.5%	57—37.5%	117—49.2%	121—50.8%
Special material	129—80.6%	31—19.4%	60—54.5%	50—45.5%	76—47.5%	84—52.5%
The special material divided into						
) Sero-positive cases	88—78.6%	4—21.4%	38—51.4%	36—48.6%	53—47.3%	59—52.7%
Sero-negative cases	41—85.4%	7—14.6%	22—61.1%	14—38.9%	23—47.9%	25—52.1%
b) Males	68—84.0%	13—16.0%	25—45.5%	30—54.5%	35—43.2%	46—56.8%
Females	61—77.2%	18—22.8%	35—63.6%	20—36.4%	41—51.9%	38—48.1%
) Children	55—80.9%	13—19.1%	24—45.5%	29—54.7%	26—38.2%	42—61.8%
Adults	74—80.4%	18—19.6%	36—63.2%	21—36.8%	50—54.3%	42—45.7%

TABLE (cont.)

	Liver		Thymol turbidity test		
	Not palpable	Palpable	Normal	4–6 U	> 6 U
Total material	183=76.9%	55=23.1%	37=17.3%	60=28.0%	117=54.7%
Special material	120=75.0%	40=25.0%	19=12.7%	38=25.3%	93=62.0%
The special material divided into					
a) Sero-positive cases	86=76.8%	26=23.2%	9=8.4%	25=23.4%	73=68.2%
Sero-negative cases	34=70.8%	14=29.2%	10=23.3%	13=30.2%	20=46.5%
b) Males	60=74.1%	21=25.9%	10=13.0%	19=24.7%	48=62.3%
Females	60=75.9%	19=24.1%	9=12.3%	19=26.0%	45=61.6%
c) Children	43=63.2%	25=36.8%	10=15.9%	17=27.0%	36=51.7%
Adults	77=83.7%	15=16.3%	9=10.3%	21=24.1%	57=65.5%

	Serum bilirubin				Exanthema	
	< 0.6 mg%	0.6–1.0 mg%	1.0–2.0 mg%	> 2 mg%	Absent	Present
Total material	85=48.3	73=41.5%	15=8.5%	3=1.7%	202=84.9%	36=15.1%
Special material	65=46.1%	60=42.6%	15=10.6%	1=0.7%	133=83.1%	27=16.9%
The special material divided into						
a) Sero-positive cases	51=50.0%	40=39.2%	10=9.8%	1=1.0%	97=86.7%	15=13.4%
Sero-negative cases	14=35.9%	20=51.3%	5=12.8%		36=75.0%	12=25.0%
b) Males	30=41.7%	33=45.8%	8=11.1%	1=1.4%	72=88.9%	9=11.1%
Females	35=50.7%	27=39.1%	7=10.1%		61=77.2%	18=22.8%
c) Children	29=49.2%	23=39.0%	7=11.7%		56=82.4%	12=17.6%
Adults	36=43.9%	37=45.1%	8=9.8%	1=1.2%	77=83.7%	15=16.3%

	Angina			Cervical lymph glands	
	Absent	Common	Membranous	Not palpable	Palpable
Total material	46=19.3%	38=16.0%	154=64.7%	14=5.9%	224=94.1%
Special material	25=15.6%	25=15.6%	110=68.8%	7=4.4%	153=95.6%
The special material divided into					
a) Sero-positive cases	16=14.3%	15=13.4%	81=72.3%	6=5.4%	106=94.6%
Sero-negative cases	9=18.8%	10=20.8%	29=60.4%	1=2.1%	47=97.9%
b) Males	9=11.1%	11=13.6%	61=75.3%	2=2.5%	79=97.5%
Females	16=20.3%	14=17.7%	49=62.0%	5=6.3%	74=93.7%
c) Children	10=14.7%	11=16.2%	47=69.1%	0=0.0%	68=100%
Adults	15=16.3%	14=15.2%	63=68.5%	7=7.6%	85=92.4%

TABLE 2. *(cont.)*

	Axillary lymph glands		Inguinal lymph glands	
	Not palpable	Palpable	Not palpable	Palpable
Total material	85—35.7%	153—64.3%	81—34.0%	157—66.0%
Special material	47—29.4 %	113—70.6%	45—28.1%	115—71.9%
The special material divided into				
a) Sero-positive cases	34—30.4	78—69.6 %	26—23.2%	86—76.8%
Sero-negative cases	13—27.1%	35—72.9%	19—39.6%	29—60.4 %
b) Males	17—21.0%	64—79.0%	17—21.0%	64—79.0%
Females	30—38.0%	49—62.0%	28—35.4%	51—64.6%
c) Children	18—26.5%	50—73.5%	17—25.0%	51—75.0%
Adults	29—31.5%	63—68.5%	28—30.4 %	64—69.6%

	Lymphadenoma at pulmonary hilus			Prothrombin index	
	Normal	Border line	Positive	Normal	< 80
Total material	172—85.6%	6—3.0%	23—11.4%	136—74.7%	46—25.3%
Special material	124—86.7%	3—2.1%	16—11.2%	111—75.0%	37—25.0%
The special material divided into					
a) Sero-positive cases	82—83.7%	3—3.1%	13—13.3%	77—73.3%	28—26.7%
Sero-negative cases	42—93.3%		3— 6.7%	34—79.1%	9—20.9%
b) Males	62—86.1%	3—4.2%	7— 9.7%	57—75.0%	19—25.0%
Females	62—87.3%		9—12.7%	54—75.0%	18—25.0%
c) Children	57—87.7%	1—1.5 %	7—10.8%	52—82.5%	11—17.5%
Adults	67—85.9%	2—2.6%	9—11.5%	59—69.4 %	26—30.6 %

	Thrombocytes		Reticulocytes		Serum Iron	
	Normal	<140,000/mm³	Normal	<0.5‰	Normal	<60γ%
Total material	121—63.4%	70—36.6%	170—96.0%	7—4.0%	58—58.6%	41—41.4%
Special material.	90—62.1%	55—37.9%	133—95.7%	6—4.3%	47—57.3%	35—42.7%
The special material divided into:						
a) Sero-positive cases	60—57.1%	45—42.9%	95—94.1%	6—5.9%	33—55.0%	27—45.0%
Sero-negative cases	30—75.0%	10—25.0%	38—100 %	0—0.0%	14—63.6%	8—36.4%
b) Males	47—62.7%	28—37.3%	72—98.6%	1—1.4 %	23—60.5%	15—39.5%
Females	43—61.4%	27—38.6%	61—92.4%	5—7.6%	24—54.5%	20—45.5%
c) Children	43—67.2%	21—32.8%	59—96.7%	2—3.3%	17—51.5%	16—48.5%
Adults	47—58.0%	34—42.0%	74—94.9%	4—5.1%	30—61.2%	19—38.8%

TABLE — (cont.)

	AS titre		ASta titre		APn titre	
	≤ 200	> 200	≤ 1.4	> 1.4	≤ 500	> 500
Total material	88=38.6%	140=61.4%	161=70.9%	66=29.1%	121=79.1%	32=20.9%
Special material	49=31.0%	109=69.0%	110=70.1	47=29.9%	80=75.5%	26=24.5%
The special material divided into						
) Sero-positive cases	30=27.3%	80=72.7%	76=69.7%	33=30.3%	61=79.2%	16=20.8%
Sero-negative cases	19=39.6%	29=60.4%	34=70.8%	14=29.2%	19=65.5%	10=34.5%

	Fusiform bacilli + Spirochaetes (Vincent culture)		ECG		
	Absent	Present	Normal	Suspected myocarditis	Myocarditis
Total material	81=91.0%	8=9.0%	225=97.0%	3=1.3%	4=1.7%

	Proteinuria			CSF		
	Absent	Febrile	Without path. sed.	Normal	3—4 cells/mm³	≥ 5 cells/mm³
Total material	211=88.7%	26=10.9%	1=0.4%	77=47.5%	42=25.9%	43=26.5%
Special material				57=45.2	29=23.0%	40=31.7%
The special material divided into						
Sero-positive cases				40=46.0%	18=20.7%	29=33.3%
Sero-negative cases				17=43.6%	11=28.2%	11=28.2%
b) Males				28=42.4	16=24.2%	22=33.3%
Females				29=48.3%	13=21.7%	18=30.0%
) Children				28=45.9%	16=26.2%	17=27.9%
Adults				29=44.6%	13=20.0%	23=35.4%

TABLE 2. *(cont.)*

	EEG		Paul-Bunnell test	
	Normal	Pathological	Negative	Positive
Total material	103—68.2%	48—31.8%	78—34.1%	151—65.9%
Special material	81—68.1%	38—31.9%	48—30.0%	112—70.0%
The special material divided into.				
) Sero-positive cases	52—63.4%	30—36.6%		
Sero-negative cases	29—78.4%	8—21.6%		
b) Males	37—61.7%	23—38.3%	26—32.1%	55—67.9%
Females	44—74.6%	15—25.4%	22—27.8%	57—72.2%
) Children	38—64.4%	21—35.6%	23—33.8%	45—66.2%
Adults	43—71.7%	17—28.3%	25—27.2%	67—72.8%

far more frequently palpable in children than in adults. It should, however be remembered here that the spleen may be palpabl in healthy children.

The difference in frequency between the total and special material was mng nificant.

On division of the special material into sub-groups, the sero-positive and sero-negative cases were found to have almost identical frequencies and males only a slightly higher figure than females. Children on the other hand, as in the total material, exhibited a markedly higher figure than adults.

Palpable liver On division of the total material into age and sex groups it was found that children had considerably greater frequency of pathological findings than adults. When noting this fact it should be remembered that healthy children may also have palpable liver

The total material and the special material exhibited good agreement as to the frequencies.

On division of the special material into sub-groups it was found that there was a somewhat greater frequency among sero-negative cases than among sero-positive. Males and females displayed very similar figures whereas children, as in the total material, had a clearly greater frequency than adults.

Thymol t bidity test Maximum values were recorded. A thymol value definitely above the normal was found to dominate in the material.

There was a tendency towards a greater frequency of high values in the special material than in the total material.

The sub-division of the special material showed a greater frequency of high values among sero-positive than among sero-negative cases. The frequency of normal values was greater among sero-negative cases than among sero-positive. Males and females were in good agreement. Adults were found to have a somewhat higher frequency of high values than children.

Serum bilirubin Maximum values were recorded. The material exhibited a certain tendency towards slightly raised bilirubin values.

In the special material there were only 6 cases of pathological findings in this respect, all of them being sero-positive.

Serum iron The lowest value was recorded and the lowest normal value set at 60 gamma%. The frequency of serum iron deficiency was found to be comparatively great

In the total material children displayed a greater frequency of pathological results than adults, i.e. in approximately 50 % of the cases as opposed to approximately 35 % among the adults.

The total material and the special material exhibited a similar frequency distribution.

In the sub-divisions of the special material it was found that there was a tendency towards a greater frequency of serum iron deficiency among sero-positive cases than among sero-negative among females than among males and among children than among adults.

Antistreptolysin titres (AS) When the highest value for each patient was recorded, this was often, as can be expected, above the upper limit for the normal variation of single values in healthy adults in Stockholm 200.

The total material contained 27 cases in which there was such a four fold change in the titr (in 10 cases among men, 5 among boys, 7 among women and 5 among girls)

With regard to individual maximum values recorded, the special material displayed a slight tendency towards a greater frequency of elevated values than the total material and, in the special material, sero-positive cases displayed the same tendency when compared with sero-negative cases. Further division of the material with regard to antibacterial titres was not made.

In the special material 80 sero-positive patients had a raised AS and 17 of these displayed a four fold or larger titre change 29 sero-negative patients had elevated AS values, and in 5 of these there was a four fold or larger titre change.

Antistaphylolysin titres (ASta) The frequency of titres above the limit (1 4) accepted for normal variation was much lower than for AS

In the total material the frequency of elevated values was similar in the various sex and age groups. Four fold or greater titre change was found in 1 man och 1 woman.

The total material and the special material proved to have a very similar frequency of raised titres. The distribution between sero-positive and sero-negative cases in the special material was likewise very similar Only two cases displayed a four fold or greater titre change (1 man and 1 woman, both sero-positive)

Antipneumolysin titres (APn) The upper normal limit was set at 500 (204)

In the total material females were found to have a greater frequency of elevated values than males. In this material 13 cases (1 man, 2 boys, 5 women and 5 girls) had four-fold or greater titre change.

The special material had an insignificantly greater frequency of elevated titres as regards individual test values than the total material had. In the special material sero-negative cases tended to have a greater similar frequency than sero-positive.

Six of 16 sero-positive cases and 6 of 10 sero-negative cases were found to have a four fold or greater titre change.

Fusiform bacilli + Spirochaetes (Vincent culture) Giemsa-stained smears of organisms known to be present in Vincent's angina were examined microscopically in 89 instances. The frequency of positive results was low

In the sub-groups of the total material it was found that there were positive results in a little more than 13 % of adults, whereas children had very low figures.

The material was not analyzed further because of the small number of positive results. It may be mentioned, however that of the patients with positive results two men were sero-positive and one sero-negative, one woman sero-positive and two sero-negative, one girl sero-positive and one sero-negative. All belonged to the group of patients without evidence of other secondary infection.

The frequency of positive findings for this type of flora seems to vary a great deal from material to material. No less than 67 % of positive results has been observed (215)

Myocarditis This diagnosis was set by ECG analysis. The frequency was low. Criteria for diagnosis were repeated ECGs and variation between them for the individual case.

Further division into sub-groups was not made because of the small number of cases found to have myocarditis. All cases thus diagnosed belonged to the group without secondary infection and consisted of two men (one sero-negative and the other sero-positive) one woman and one girl (both sero-positive)

Three cases were referred to the group of suspected myocarditis. The necessary control examinations were not performed on them.

Proteinuria With the exception of proteinuria which appeared during the pyretic period, only one instance was observed. This was a 6-year-old boy sero-positive and belonging to the group without secondary infection. His urine was quite free from protein before he left the hospital.

In no instance was any pathological sediment found.

Thus no case of serious kidney complication occurred in the material.

Cerebro-spinal fluid (CSF) CSF findings on lumbar puncture were judged by the appearance of cells. In this regard cases which were found to have 5 cells or more per mm³ were deemed clearly pathological, cases with 3—4 cells per mm³ doubtfully pathological, and cases with 0—2 cells per mm³ normal. In this material, as in previous series (157) it was observed that there were cases with a pathological number of cells in the CSF without symptoms of meningitis.

23 % of the total material were found to belong to the doubtful intermediate group and a slightly greater frequency to the pathological group.

The special material exhibited a somewhat greater frequency of clearly pathological cases than the total material, while the situation was found to be reversed in the doubtful group

In the special material sero-positive cases tended to have a greater frequency of clearly pathological results than sero-negative, whereas the relationship in the doubtful group was reversed. Males showed a slight tendency towards greater frequencies both in the clearly pathological as well as in the doubtful group. Adults displayed a greater frequency of pathological results than children, this situation being opposite to that found in the doubtful group.

Electroencephalogram (EEG) In the total material and the special material pathological EEGs were found in almost 1/3 of the cases examined in this respect. The frequency was almost exactly the same in both these groups of the material.

In the total material the frequency of pathological EEGs ranged from 23.1 % among women to 43.6 % among boys.

In the special material the tendency was found to be that sero-positive cases had a greater frequency of pathological EEGs than sero-negative males a greater frequency than females and children a somewhat greater frequency than adults.

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sages of tissue culture fluid from these samples to similar cultures did not give rise to any degenerative changes, however Thus, no cytopathogenic agents were de monstrated.

Summary

The section of the entire material which is referred to in Table 2 as the special material was found to have a slight tendency towards a greater frequency of pathological symptoms than the total material. The special material consists of those cases on which a special cytological examination was performed.

In the sub-groupings of the special material it was found that sero-positive cases often tended to have a greater frequency of pathological symptoms than sero-negative. As regards the division into adults and children and males and females it was found that adults tended to exhibit a greater frequency of pathological results of thymol, prothrombin and thrombocyte tests and tended to have a longer duration of pyrexia than children.

Children, on the other hand, tended to ha e a greater frequency of palpable spleen and liver nnuitis, anaemia and serum iron deficiency than adults. Females tended to ha e a greater frequency of anaemia, serum iron deficiency oedema of the eyelids and exanthema than males, whereas the situation was reversed as regards sinu tis and pathological EEG

All differenc s were moderate S nce the n mber of test vari d the table only rves th object of pr se t ng the results f re orded maximum and minimum val es d cannot be us d as a basis for nequivocal statistical analysis It is nly intended for the demo st atio f the cli ical background of the material f m whi h th cytological st dies i this work originate

b. Findings of Special Interest· Greatest Relative Frequency of Atypical Cells the Value of First and Greatest ESRs, the Diagnostic Significance of the Serological Reaction from the Clinical Point of View

Table 3 shows the distribution in the special material of the greatest frequency of atypical cells by differential count. The material is divided into two groups without and with secondary infection, i.e. of proved bacterial and viral infection.

Th total number of these cases is 165 this being du to the fact that 5 cases on which the Paul-Bunnell test was not perform ed are included. These cases were excluded from the main report on the special material.

The frequencies have been grouped in accordance with the column headings of the table. The table presents the number of patients belonging to the various groups and the percentage distribution within each age and sex group

The table is included because of a general interest in showing the maximum recorded values of the occurrence of atypical cells. It can have no claim as a basis for statistical analysis since the number of tests aried from patient to patient

The test records as such show that almost half the cases in Group I at least once had a relative frequency of atypical cells of 15 % or more. Th results in Group II indicate that not quite 1/3 had a relative frequency of 15 % or more and that 1/3 had 4—7.5 % as a greatest alue. When both groups are taken together it is found that 44 2 % of cases had a maxi mum relative frequency for the atypical cells of 15 % or more.

Table 4 show the arithmetic mean of the first ESR test after the arrival of the individual patient t the hospital and the

TABLE 3. *Greatest relative occurrence of atypical cells by differential count (special material)*

Relative frequency	0–3.5%		4–7.5%		8–9%		9.5–11.5%		12–14.5%		15–100%		No.
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	Total
Group I Cases without secondary infection.													
Men	1	2.3	7	16.3	4	9.3	4	9.3	3	7.0	24	55.8	43
Boys	4	13.3	7	23.3	2	6.7	1	3.3	4	13.3	12	40.0	30
Women	3	9.7	6	19.4	6	19.4	1	3.2	1	3.2	14	45.2	31
Girls	2	7.1	4	14.3	6	21.4	1	3.6	2	7.1	13	46.4	28
Total	10	7.6	24	18.2	18	13.6	7	5.3	10	7.6	63	47.7	132
Group II Cases with secondary infection.													
Men	—	—	1	33.3	—	—	—	—	—	—	2	66.7	3
Boys	—	—	4	44.4	2	22.2	1	11.1	—	—	2	22.2	9
Women	2	15.4	3	23.1	1	7.6	2	15.4	1	7.6	4	30.8	13
Girls	1	12.5	3	37.5	—	—	2	25.0	—	—	2	25.0	8
Total	3	9.1	11	33.3	3	9.1	5	15.2	1	3.0	10	30.3	33

arithmetic mean of the time which had passed from onset of disease to this test for each individual patient. The series, which consists of the total material, is divided into two groups, cases *without* and cases *with* secondary infection. The intermediate group in this respect doubtful, which in the total material consisted of 3 cases, has been excluded. The table first presents the total numbers in both groups and then divides them into males and females and finally into children and adults, the upper age limit for children being up to and including the age of 14

The total number of cases within the groups displays good agreement both as regards ESR values and the time between onset and testing. When the material is divided into its sub-groups it is found that there is very moderate spread both regarding the ESR as the time factor

Table 5 shows in a similar manner the greatest ESR values recorded.

The mean of maximal ESRs lies within reasonable limits. The difference between the mean of the first and the greatest ESR is moderate in size. The greatest ESR is reached fairly early on during the course of the disease.

It should be emphasized here that the table showing the greatest ESR values cannot be used as a basis for statistical analysis. It contains only arithmetic means of the records of greatest ESR results. The number of tests performed varied from patient to patient.

On the basis of the results obtained in this study an attempt has been made to arrive at an answer to the question as to whether a positive serological reaction is necessary for diagnosing a case of infectious mononucleosis or not, for this is a question of much importance and it has been discussed a great deal (18 51 62, 66, 81 96, 97 98, 107 118, 120 131 148 177 184 186, 197 198)

In order to approach this problem, several symptoms characteristic of infectious mononucleosis were chosen from the special material namely the simultane-

TABLE 4 *First ESR (total material)*

	No.	Mean ESR (mm)	Mean time (days)
Group I. Cases without secondary infection.			
Total	189	25.6	9.2
Males	108	24.0	8.7
Females	81	27.9	9.9
Children	73	29.4	8.7
Adults	116	23.3	9.6
Group II Cases with secondary infection.			
Total	46	23.5	9.4
Males	20	25.7	6.8
Females	26	23.3	11.5
Children	24	23.7	7.9
Adults	22	27.4	11.1

Time = Period from onset of disease to first ESR test.

TABLE 5. *Greatest ESR (total material)*

	No.	Mean ESR (mm)	Mean time (days)
Group I Cases without secondary infection.			
Total	189	31.6	13.3
Males	108	28.3	12.1
Females	81	33.8	15.0
Children	73	32.2	12.7
Adults	116	31.2	13.7
Group II Cases with secondary infection			
Total	46	31.6	14.0
Males	20	31.4	12.4
Females	26	31.7	15.3
Children	24	29.9	13.9
Adults	22	33.5	14.2

Time = Period from onset of disease the ESR test in question.

TABLE 6. *Percentage frequencies from the special material as distributed in sero-positive and sero-negative cases. (The frequencies calculated among 112 sero-positive and 48 sero-negative cases)*

a. Palpable cervical, axillary and inguinal lymph glands

Sero-positive cases.	72 = 64.3 %
Sero-negative cases	28 = 58.3 %

b Palpable cervical, axillary and inguinal lymph glands in conjunction with membranous angina

Sero-positive cases.	57 = 50.9 %
Sero-negative cases	20 = 41.7 %

Palpable cervical, axillary and inguinal lymph glands in conjunction with palpable spleen

Sero-positive cases	44 = 39.3 %
Sero-negative cases	17 = 35.4 %

d Palpable cervical, axillary and inguinal lymph glands in conjunction with pyrexia

	Pyrexia			
	1 day—1 week	1—2 weeks	2—3 weeks	3 weeks and >
Sero-positive cases	17 = 23.6 %	34 = 47.2	16 = 22.2 %	5 = 6.9 %
Sero-negative cases	8 = 28.6 %	14 = 50.0 %	4 = 14.3 %	2 = 7.1 %

ous occurrence of palpable lymph glands in the cervical, axillary and inguinal regions, the occurrence of membranous angina and palpable spleen, and the duration of pyrexia. These were then contrasted in different ways as shown in T ble 6 a, b, c and d.

Result When regard is paid to the symptom complex simultaneous oc currence of palpable cervical, axillary

and inguinal lymph glands in conjunction with membranous angina, and the same complex in conjunction with palpable spleen, the percentage distribution of the various combinations of symptoms is somewhat higher in the sero-positive group. However the same complex of symtoms appears in such a great frequency among the sero-negative cases that *the result of the serological reactions* cannot be regarded as a deciding factor

With regard to the symptom of pyrexia, the result according to Table 2 (duration of pyrexia without regard to other symptoms) is referred to Here it is shown that 25 % of the sero-positive cases still exhibit pyrexia after two weeks, whereas this is so in 22.9 % of the sero-negative cases. Thus the frequency in the sero-negative group is somewhat lower but the difference very small.

If regard is paid to the duration of pyrexia in cases with a simultaneous occurrence of palpable cervical, axillary and inguinal lymph glands alone, then the frequency of cases still pyretic after two weeks is 29 1 % among the sero-positive and 21 4 % among the sero-negative. Thus at least each 5th sero-negative case is still pyretic *after two* weeks regardless of whether the aforementioned glandular symptoms occur simultaneously or not. Thus, as with palpable lymph glands, membranous angina and palpable spleen, is nevertheless a clear symptom of the disease further indicating that the serological reaction cannot be of decisive importance.

The study does not therefore support the suggestion that a positive result of the serological test is necessary for the diagnosis of infectious mononucleosis On the other hand it does show that the sero-positive cases suffer more seriously from the disease.

CHAPTER 4

Statistical Analysis of the Haematological Material

a. Relative Frequency and Absolute Numbers of the Different White Blood Corpuscles

Information on the leukocytes refers in each individual case to the first blood sampling to be included in the special investigation and constitutes the number of total leukocytes and a differential count made on 200 cells. Arithmetic means of the number of blood cells belonging to the various types were then calculated from this starting point. The differential count was carried out according to the criteria stipulated in the Introduction and the chapter on Cytology. The material is first dealt with in its entirety. It is then divided into sero-positive and females and finally into adults and and sero-negative groups, into males children (Tables 7—8).

The Special Material

	Sero-positive	Sero-negative
Ma	33	13
Mc	22	13
Fa	34	12
Fc	23	10

Ma = Male adults F = Female adults
Mc = Male children F = Female children

Table 7 contains information on the first blood sampling of the individual patient after admittance to hospital, and presents the mean values of the results of the differential count. As regards the whole of this particular material it exhibits a marked lymphocytosis — 50 % lymphocytes and 17.7 % atypical cells. Lymphocytic and monocytic cells (lymphocytes, atypical cells, monocytes and plasma cells) together amount to 70.2 %. The distribution among the leukocytes as a whole shows a relatively somewhat high figure of 6 % for the rod-nucleated leukocytes.

When the material is divided into sero-positive and sero-negative cases, it is found that the sero-positive have a slightly greater frequency (71.4 %) of lymphocytic and monocytic cells, whereas the corresponding figure for the sero-negative cases is 66.2 %. Both atypical cells and lymphocytes display a greater relative frequency among sero-positive cases than among the sero-negative. The sero-negative cases have a slightly higher figure for segmented leukocytes than the sero-positive.

It is important to note that the lymphocytic reaction is greater among the sero-positive cases than among the sero-negative.

The division into males and females shows a 4 % units greater relative occurrence of atypical cells among the males than among the females. However the relative frequency of lymphocytes among the females is greater so that the difference is reduced to 2 % units if atypical cells and lymphocytes are taken together and to 1.8 % units if all lymphocytic and monocytic cells are taken into account. The more powerful reaction with the marked relative over-measure of atypical cells among the males is noteworthy. Differences on the leukocytic side are very small and chiefly limited to a slightly greater relative frequency of the common segmen-

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TABLE 7 *Differential count. The distribution in mean percentages*

No.	R leuko-cytes	S leuko-cytes	Eosino-phils	Baso-phils	Lympho-cytes	Mono-cytes	Plasma-cells	Atypical cells
160 Total	6.0	22.5	1.0	0.3	50.0	2.3	0.2	17.7
112 Pos. PB	5.5	21.4	0.9	0.3	50.8	2.1	0.2	18.8
48 Neg. PB	7.1	24.9	1.4	0.4	48.0	2.9	0.2	15.1
81 Males	3.9	21.8	0.8	0.3	49.8	2.2	0.2	19.7
79 Females	6.0	23.2	1.2	0.4	51.0	2.4	0.2	15.7
68 Children	6.1	24.3	1.2	0.3	50.6	2.5	0.2	14.4
92 Adults	5.9	21.0	0.9	0.3	49.4	2.2	0.2	20.1

ted leukocytes among the females (1.4 % units) thus for the most part corresponding to the difference in the lymphocytic blood picture.

Finally the division into adults and children displays an almost 6 % units greater relative occurrence of atypical cells in the adults as opposed to the children. When all lymphocytic, monocytic and plasmacellular cells are taken together the difference in relative frequencies is 4 % units, the adults still having the advance. Distribution on the leukocytic side is ordinary in relation to the other groupings of the material.

Result

Differential count of the special material is reported on with a presentation of the arithmetic means of the results for the material as a whole as well as for results after division of the material according to serum reaction, sex and age.

Differential count showed that there was a pointed lymphocytic reaction in the material with a great relative occurrence of atypical cells and a nominal frequency of about 50 for the lymphocytes. The percentage of the neutrophils in the blood picture was correspondingly reduced but this reduction was due to the common segmented leukocytes alone. Eosinophile and basophile leukocytes were present in normal relative numbers. The occurrence of the rod-nucleated leukocytes was of an insignificantly greater relative frequency.

Discussion

Three factors are prominent in the table.

1) The relative superabundance of lymphocytic cells. This is very marked, with lymphocyte values of about 50 % in all groupings of the material. It may thus be observed in the last section of the table how the adults also exhibit severe lymphocytosis—this is therefore not a question of a blood picture of lymphocytic type for any specific age, particularly that of childhood.

The sero-negative cases display relative lymphocytosis of high degree even though the frequency in their instance is somewhat less than that of the sero-positive cases. Likewise, both sexes display a high frequency figure.

2) The great relative frequency of atypical cells within the various groups. Between the groups however there is a certain difference with a greater relative frequency among sero-positive cases than among the sero-negative. Similarly

ery small. The central group, 5—9 years, consists of 32 children, however indicating that the juvenile material is not dominated by the older children but is evenly distributed between the ages of 5 and 14. The serum reaction in this juvenile material is positive to a high degree and cannot be of any importance as a cause of the lower occurrence of atypical cells among children. It seems that this is in all probability due to the age factor itself.

In this connection, however it should finally be said that the difference in question is of a very low order and is not statistically significant (cf. the following section dealing with the statistical analysis). Nevertheless, the interesting fact remains that the juvenile material, which in itself exhibits a normal tendency towards lymphocytosis, has no more marked a tendency in infectious mononucleosis than appears from the results of this study.

Results

Table 8 illustrates the absolute numbers of cells from the first sampling expressed as geometric means and with the material grouped according to the same principle as that employed in Table 7. The table contains information on the numbers of total leukocytes, lymphocytes, typical cells, monocytes and R & S leukocytes.

The table indicates a moderately increased number of total leukocytes and clearly illustrates the same relationships which the previous table presented in relative numbers, i.e. marked lymphocytosis with a greater number of lymphocytes than R & S leukocytes and the marked occurrence of atypical cells which occur in greater numbers than the monocytes.

The material is divided into the same groups as in Table 7. The number of total leukocytes is greater among the sero-positive cases than among the sero-negative, greater among males than females and among adults than among children.

There are greater numbers of atypical cells among the sero-positive cases than among the sero-negative, among males than among females and among adults than among children.

Discussion

Conspicuous in this table is the great occurrence of lymphocytes and atypical cells among a number of total leukocytes which slightly exceeds the upper limit of what is generally considered to be normal.

The number of R & S leukocytes lies at the lower normal limit and cannot definitely be considered to be influenced.

In the various groupings of the material there are certain differences which in absolute form reflect the relative differences which appeared in the table dealing with the results of the differential count (Table 7).

Regarding the lymphocytes, it appears that when calculated in absolute numbers there is a dominant figure for sero-positive cases, males and adults, as opposed to the respective sero-negative cases, females and children.

More conspicuous is the excess of atypical cells among the sero-positive cases as opposed to the sero-negative to a lesser extent among males as opposed to females and among adults as opposed to children.

b. Graphic Analysis of the Material

The material forming the basis for the following graphic presentation consists of cases without secondary infection which were examined haematologically within

number of R & S leukocytes. There is thus clear dominance on the lymphocytic and monocytic side. The total number of leukocytes hardly amounts to 9,000 per mm³

The first section deals with the division of the material into sero-positive and sero-negative cases. All cell types with the exception of the monocytes occur in greater numbers among the sero-positive cases than among the sero-negative, even though the difference as regards the R & S leukocytes is of little account, and moderate as regards the lymphocytes. The monocytes occur in slightly greater numbers among the sero-negative cases than among the sero-positive. The difference between the two patient groups with regard to the atypical cells is great.

It would be of interest if the combined totals of lymphocytic and monocytic cells were similar in both the groups of the material. It appears regarding the combined totals of these cells, however that there is a difference between the groups, the sero-positive cases being responsible for the larger figure.

The distribution among males and females irrespective of the serum reaction shows a greater number of leukocytes totally among the males.

All the various types of leukocytes occur in larger numbers among the males than among the females. The atypical cells exhibit the greater difference whereas the difference for the remainder is moderate (lymphocytes and R & S leukocytes) or slight (monocytes). The lymphocytic and monocytic cells taken together have a greater occurrence among the males.

The lowest section of the table divides the material into adults and children. The number of total leukocytes is somewhat greater among the adults than among the children. Both lymphocytes and atypical cells occur to a greater extent among the adults than the children. The situation is reversed as regards the monocytes and the R & S leukocytes. All mononuclear cells (lymphocytes, atypical cells and monocytes) taken together are found to have only a slightly higher figure for the adults than for the children. The atypical cells by themselves on the other hand, show a moderate difference between the two groups.

The relative lymphocytosis among the adults in the material is marked. The lesser occurrence of lymphocytic cells among the children than among the adults seems at first sight to be puzzling since it is known that children usually show a relatively greater frequency of lymphocytes than adults. However it should be borne in mind that the children in this material comprise all cases up to and including the age of 14. There are only three cases in the youngest age group of 0—4 years yet 33 in the oldest of 10—14 years. In such a juvenile material with so few children in the youngest age group it is thus not normally to be expected that there will be a high relative lymphocytosis.

Here it has been possible to show that sero-positive cases have a greater number of atypical cells than sero-negative cases. It therefore seems fairly natural to see this as an explanation as to why the adults have a greater number of atypical cells than the children, since it is generally stated that adults exhibit a greater frequency of sero-positivity than children. However the frequency of sero-positivity in this material is 72.8 % among adults and 66.2 % among children, i.e. only a relatively slight difference. A contributing factor to this may be that the number of children in the youngest age group is

very small. The central group, 5—9 years, consists of 32 children, however indicating that the juvenile material is not dominated by the older children but is evenly distributed between the ages of 5 and 14. The serum reaction in this juvenile material is positive to a high degree and cannot be of any importance as a cause of the lower occurrence of atypical cells among children. It seems that this is in all probability due to the age factor itself.

In this connection, however it should finally be said that the difference in question is of a very low order and is not statistically significant (cf. the following section dealing with the statistical analysis). Nevertheless, the interesting fact remains that the juvenile material, which in itself exhibits a normal tendency towards lymphocytosis, has no more marked a tendency in infectious mononucleosis than appears from the results of this study.

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More conspicuous is the excess of atypical cells among the sero-positive cases as opposed to the sero-negative to a lesser extent among males as opposed to females and among adults as opposed to children.

b. Graphic Analysis of the Material

The material forming the basis for the following graphic presentation consists of cases without secondary infection which were examined haematologically within

the first fourteen days after onset of disease. In all they amount to 94 cases and are distributed as follows

	Sero-positive cases	Sero-negative cases
Ma	18	12
Mc	15	8
Fa	13	6
Fc	15	7

The initial values for the numbers of total leukocytes, lymphocytes, atypical cells, monocytes and R & S leukocytes expressed as a logarithm for the whole of this patient group divided into sero-positive and sero-negative cases alone are as follows

	Sero-positive cases, log	Sero-negati cases, log
Leukocytes	4 02	3 89
R & S leukocytes	3 42	3.36
Lymphocytes	3.67	3.51
Monocytes	2.01	2 14
Atypical cells	3.14	2.84

In the efforts to present this material graphically a number of difficulties were met with which are gone into in greater detail in the discussion of this chapter In spite of this, it was believed to be of value to try to arrive at a simple visual presentation of the quantitative course of the whit blood corpuscles during the disease thus being the reason for the inclusion of the diagrams, which were drawn up according to the seemingly best method available.

Since samplings were taken on different days from the onset of disease in the various patients, and since mean values at certain time intervals were wanted, a value for each such time interval required was interpolated for each patient. This was done for each of the first 10 days after onset of disease, and after this for each 4th day Table 9 illustrates these recording days and the logarithmic means in question for the various cell types.

Primary scrutiny of the graphs first drawn up exhibited little difference between the various age and sex groups as regards the different types of cells. Because of this it was only considered necessary to go into detail regarding the graphs of larger patient groups. They illustrate

A The quantitative cours of the tot l leukocytes R & S leukocytes lymphocytes atypical cells and monocytes in

a) Sero-positive cases (fig 2 a)

The number of total leukocytes exhibits only a slight tendency to fall.

Of the curves of the 4 cell types, that of the lymphocytes remains highest right up until almost the end of the diagram, where it is cut by that of the R & S leukocytes. The R & S leukocyte curve remains at a very moderate distance below the lymphocyte curve until the point of cutting with the lymphocyte curve just mentioned. The atypical cells begin at almost the same level as the R & S leukocytes and then fall off more or less evenly until about the 20th day of disease, after which the falling-off is only slight. The monocyte curve remains at a comparatively low level the whole time and is generally speaking flat with only a slight tendency to rise.

b) Sero-negative cases (fig 2 b)

Observation of the sero-negative cases ended on the 38th day

The number of total leukocytes exhibits only a slight tendency to rise.

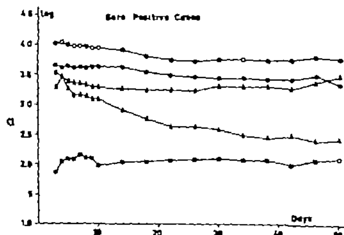
The lymphocytes and R & S leukocytes were found to begin at practically

the same point. However the lymphocytes thereafter lie just above the R & S leukocytes the whole time. Both curves are principally horizontal. The atypical cells lie at a greater distance below the R & S leukocytes — always above the monocytes however — rise up to the 10th day of disease but then very gradually fall off relatively evenly until the end-point lies somewhat higher than the starting point. The monocytes, which exhibited the lowest frequency of cells during the entire period, were found — regardless of smaller variations in the beginning of the curve — to present a rather flat curve.

B. Graphic comparison between the quantitative course in sero-positive and sero-negative cases of

a) The number of total leukocytes (fig. 3 a)

The curve for the sero-negative cases begins somewhat lower than that for the sero-positive, but converges on it before the 10th day of disease. After this it lies in close proximity to it.



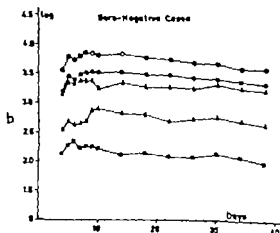


Fig. 2 and b. Quantitative Course of Various White Blood Corpuscles in Sero-Positive and Sero-Negative Cases.

Total leukocytes
● Lymphocytes
△ R & S leukocytes
▲ Atypical cells
☐ Monocytes

the first fourteen days after onset of disease. In all they amount to 94 cases and are distributed as follows

	Sero-positive cases	Sero-negati e cases
Ma	18	12
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A The quantitative course of the total leukocytes R & S leukocytes lymphocytes atypical cells and monocytes in

a) Sero-positive cases (fig 2 a)

The number of total leukocytes exhibits only a slight tendency to fall

Of the curves of the 4 cell types, that of the lymphocytes remains highest right up until almost the end of the diagram, where it is cut by that of the R & S leukocytes. The R & S leukocyte curve remains at a very moderate distance below the lymphocyte curve until the point of cutting with the lymphocyte curve just mentioned. The atypical cells begin at almost the same level as the R & S leukocytes and then fall off more or less evenly until about the 20th day of disease, after which the falling-off is only slight. The monocyte curve remains at a comparatively low level the whole time and is generally speaking flat with only a slight tendency to rise.

b) Sero-negative cases (fig 2 b)

Observation of the sero-negative cases ended on the 38th day

The number of total leukocytes exhibits only a slight tendency to rise.

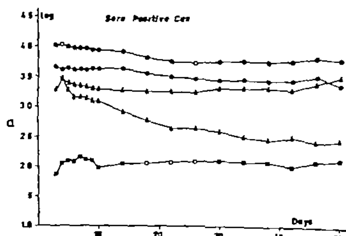
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the same point. However the lymphocytes thereafter lie just above the R & S leukocytes the whole time. Both curves are principally horizontal. The atypical cells lie at a greater distance below the R & S leukocytes — always above the monocytes however — rise up to the 10th day of disease but then very gradually fall off relatively evenly until the end-point lies somewhat higher than the starting-point. The monocytes, which exhibited the lowest frequency of cells during the entire period, were found — regardless of smaller variations in the beginning of the curve — to present a rather flat curve.

B Graphic comparison between the quantitative course in sero-positive and sero-negative cases of

a) The number of total leukocytes (fig 3 a)

The curve for the sero-negative cases begins somewhat lower than that for the sero-positive, but converges on it before the 10th day of disease. After this it lies in close proximity to it.



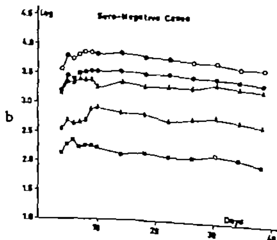
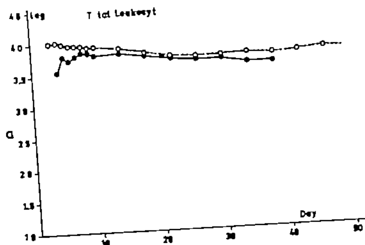
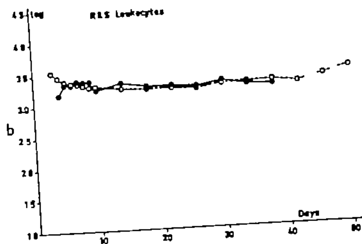
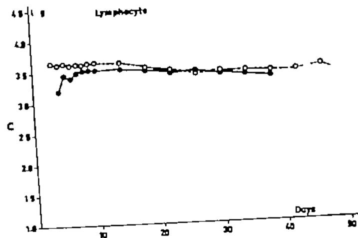


Fig 2 a and b. Quantitative Course of Various White Blood Corpuscles in Sero-Positive and Sero-Negative Cases.
○ Total leukocytes
● Lymphocytes
△ R & S leukocytes
▲ Atypical cells
□ Monocytes


T tal Leukocyt
Day
a


RES Leukocytes
Days
b


Lymphocyte
Days
c

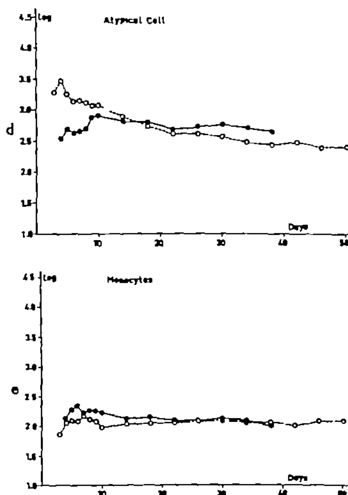


Fig 3 —e. Graphic Comparison between Sero-Positiv and Sero-Negative Cases with Regard to the Quantitative Course of Various White Blood Corpuscles.
○ Sero-positive cases
● Sero-negative cases

b) R & S leukocyt s (fig 3 b)
The curves lie extremely close to each other

) Lymphocyt (fig 3 c)
The curve of the sero-negative cases begins at a slightly lower level than that of the sero-positive closes in on it ery rapidly and thereafter lies in close proximity to it.

d) Atypical lls (f g 3 d)
The sero-negative case curve begins t a considerably lower level than the sero-positive. The curves approach each other on the 10th day of disease and soon the sero-negative curve crosses that of the sero-positive and lies slightly above it. The sero-positive case curve exhibits a moderate decline up to about the 20th

TABLE 9 *Interpolated mean value of the total leukocytes rod-nucleated and segmented leukocytes lymphocytes monocytes and atypical cells on different days after onset of disease These values form the basis for the graphs. Log value*

Day after onset	N	Total leukocytes $\bar{x}$	Range	R & S leukocytes $\bar{x}$	Range	Lymphocytes $\bar{x}$	Range	Monocytes $\bar{x}$	Range	Atypical cells $\tilde{x}$	Range	N
a. Sero-positive cases												
3	3	4.020	0.26	3.530	0.17	3.647	0.11	1.867	1.34	3.277	0.77	3
4	7	4.031	0.43	3.456	0.54	3.611	0.65	2.046	1.52	3.471	0.83	7
5	13	3.999	0.44	3.382	0.48	3.633	0.90	2.087	1.33	3.262	1.04	13
6	17	3.967	0.66	3.348	0.47	3.608	1.12	2.077	1.52	3.141	1.90	17
7	20	3.970	0.69	3.349	0.56	3.622	1.01	2.1[illegible]6	1.24	3.152	1.53	20
8	26	3.958	0.64	3.316	0.82	3.610	1.06	2.110	1.48	3.125	1.47	26
9	28	3.938	0.62	3.281	0.70	3.626	0.90	2.083	1.39	3.081	1.20	28
10	34	3.936	0.64	3.291	0.81	3.633	1.00	1.975	1.36	3.079	1.37	34
14	51	3.904	0.75	3.252	0.87	3.624	0.98	2.034	1.66	2.898	1.71	50
18	43	3.810	0.59	3.235	1.08	3.541	0.83	2.041	1.44	2.755	1.80	42
22	39	3.754	0.62	3.232	0.79	3.482	0.70	2.066	1.38	2.619	1.72	39
26	30	3.729	0.74	3.223	1.02	3.463	0.68	2.078	1.30	2.622	1.80	29
30	21	3.736	0.70	3.288	0.96	3.430	0.66	2.088	1.24	2.580	1.20	20
34	18	3.745	0.65	3.293	0.87	3.439	0.62	2.071	1.16	2.478	1.38	18
38	17	3.732	0.63	3.297	0.97	3.420	0.60	2.070	1.06	2.436	1.28	16
42	10	3.733	0.52	3.262	1.33	3.415	0.62	2.008	1.04	2.486	1.17	9
46	8	3.795	0.52	3.371	0.61	3.490	0.75	2.078	1.02	2.401	1.07	7
50	4	3.770	0.40	3.473	0.51	3.350	0.50	2.093	0.99	2.420	0.93	4
b. Sero-negative cases												
3												
4	2	3.560	0.06	3.170	0.24	3.185	0.37	2.135	0.00	2.540	0.42	
5	8	3.785	0.72	3.340	0.76	3.454	0.79	2.278	1.46	2.701	1.76	8
6	8	3.738	0.71	3.343	0.72	3.596	0.51	2.331	0.97	2.625	1.84	8
7	12	3.799	0.87	3.383	0.68	3.484	1.08	2.225	1.44	2.650	1.88	12
8	15	3.850	0.83	3.367	0.69	3.524	1.05	2.264	1.67	2.691	2.11	15
9	18	3.844	0.78	3.384	0.95	3.526	0.94	2.257	1.44	2.877	2.10	17
10	18	3.811	0.72	3.244	1.64	3.517	0.86	2.222	1.51	2.911	2.06	17
14	23	3.833	0.66	3.341	0.89	3.328	0.96	2.124	1.47	2.817	2.32	23
18	16	3.768	0.47	3.268	0.85	3.486	0.76	2.150	1.40	2.810	1.57	15
22	13	3.733	0.43	3.281	0.55	3.479	0.61	2.090	1.28	2.669	1.56	13
26	11	3.685	0.38	3.249	0.60	3.428	0.39	2.076	1.25	2.732	1.09	11
30	8	3.684	0.48	3.318	0.32	3.424	0.37	2.135	0.95	2.769	1.00	7
34	6	3.605	0.42	3.260	0.44	3.380	0.31	2.092	0.93	2.710	0.43	4
38	5	3.604	0.47	3.232	0.43	3.342	0.25	1.998	1.04	2.647	0.46	3

N = number of observations.

$\bar{x}$ refers to total leukocytes, lymphocytes, monocytes and R & S leukocytes.

$\tilde{x}$ refers to atypical cells.

day after onset, after which it flattens out. The sero-negative case curve has a slight tendency to rise.

) monocytes (fig. 3 e)

The curves lie very close to each other. Their primary tendency is to maintain a level course.

Summarizing Description of the Graphs

Observation of the sero-negative patients ceased on the 38th day.

Recording of results was made on each of the first 10 days, this being the explanation for the initial irregularity in the curves.

The graph for the atypical cells in the sero-positive patients displayed a clear falling tendency. The main tendency in all the other diagrams was for the curves to maintain a flat course in an almost horizontal position.

Discussion

The graphs which have been described are embarrassed by several weaknesses. This will be indirectly understood from the description of the interpolation and from the table on which they are based. Thus the number of readings varied from one day of recording to another whereby the starting points of the diagrams are regularly represented by a low number of patients. This explains the discrepancies occurring between the starting points of the graphs and the logarithmic values for the various cell types among sero-positive and sero-negative cases which were presented in tabular form at the beginning of this chapter. The discrepancies are inappreciable, however.

A further weakness concerning the graphs is the recordings from the 11th day on, since these, for practical reasons, were made only every 4th day (see the technical description) and are thus of necessity somewhat inexact.

There is also a weakness regarding the curves for the later days of observation when the number of cases taking part in the investigation has once again fallen off. There is therefore a risk here that the curves are mainly represented by the most severe cases of the disease. This can be compensated for to a certain extent, however, by the inclusion of available out-patient follow-up results.

On the other hand, the curves do give a clear visual impression of the quantitative course of the various cell types during the first stage of disease, and therefore should be of value as a guide provided that the reader limits himself to the larger groupings of the material as has been done in the presentation.

Regarding the atypical cells, the sero-positive cases preponderate over the sero-negative initially, illustrating the statistically established difference between these groups in this respect (Chapter 4 c). It is of much interest to note that the curves approach each other comparatively swiftly from the 9th day on, lie close together and then cross on the 16th.

This exemplifies the importance of early testing and that it is only by such a method that it is possible to obtain an established difference between sero-positive and sero-negative cases as regards the frequency of atypical cells.

Of all cell types, it is only the curve for the atypical cells which does not show signs of stabilization during the period of observation. Here a continued analysis would thus have been of value, but for practical reasons this was unfortunately impossible to do since the patients otherwise achieved complete clinical recuperation.

TABLE 10. *Presentation of analysis of significance of the differences between children/adults males/females and sero-positive/sero-negative cases with regard to the number of total leukocytes and lymphocytes. Log values*

	No. of cases	Total leukocytes			Lymphocytes		
		Mean and standard error	Standard deviation	P Evaluating the signifi-cance of the differences between means	Mean and standard error	Standard deviation	P Evaluating the signifi-cance of the differences between means
Children	68	3.94 ± 0.03	0.21	0.44	3.61 ± 0.04	0.33	0.00
Adults	92	3.96 ± 0.02	0.20		3.61 ± 0.03	0.27	
Males	81	3.99 ± 0.02	0.19	4.97*	3.64 ± 0.03	0.27	1.25
Females	79	3.91 ± 0.02	0.22		3.58 ± 0.04	0.32	
Sero-positive cases	112	3.97 ± 0.02	0.19	3.40	3.64 ± 0.03	0.25	3.34
Sero-negative cases	48	3.91 ± 0.03	0.23		3.55 ± 0.05	0.35	
Total	160	3.95 ± 0.02	0.20		3.61 ± 0.02	0.30	

Non-significant.
Denotes $P < 0.05$.

In this connection it should be pointed out that the literature contains references which state that the atypical cells can persist for as long as a year — at times even for several years — after the disappearance of other symptoms of the disease (38, 68, 86, 117). With our present knowledge of the appearance of these cells in most varied states of disorder as well as in healthy individuals (cf. Chapter 7) it seems to be pointless to try to discover the time of disappearance of the last atypical cells. What is of importance for the diagnosis of the individual case however is the presence, preferably of an increased number of typical cells *in conjunction with other clinical symptoms*. The lower limit for the pathological occurrence of atypical cells during the course of infectious mononucleosis cannot at the present stage be decided on.

A further sign showing that the disease was haematologically inadequate is the persisting lymphocytosis. This is best exemplified in the Group A graphs.

Patients who came under observation after two weeks or more had elapsed since onset are not included in this section of the investigation. The reason for this is that when it was attempted to draw up graphs for them it was found that the curves were irregular with marked fluctuations, especially as regards lymphocytes and atypical cells. Moreover the curves for these types of cells were regarding such patients, generally higher than the corresponding mean curves for

TABLE 11 *Result of correlation analysis in combination with covariance analysis between total leukocytes and atypical cells in the entire present material and its various groups. Log values*

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
			Atypical cells					t	F	F	F
	No. of cases	Total leukocytes (mean)	Mean and standard error	Standard deviation	Corrected standard deviation	Coefficient of regression	Coefficient of correlation	Evaluating the significance of the correlation			
Children	68	3.94	2.91±0.06	0.49	0.46	0.880	0.382	3.36	3.5	3.10	0.76
Adults	92	3.96	3.07±0.06	0.55	0.50	1.205	0.434	4.56			
Males	81	3.99	3.08±0.06	0.54	0.51	0.925	0.321	3.02	4.34	1.64	1.07
Females	79	3.91	2.91±0.06	0.52	0.46	1 110	0.470	4 67			
Sero-positive cases.	112	3.97	3.08±0.05	0.49	0.45	0.964	0.376	4.25	8 59	6 09	0.09
Sero-negative cases.	48	3.91	2.81±0 08	0.58	0.53	1.081	0.428	3.21			
Total	160	3.95	3.00±0 04	0.53	0.49	1.073	0.415	5.72			

Evaluating the significance { in F of the difference between unstandardized means, in F of the difference between standardized means and in F of the difference between regression coefficients.

Non-significant.

Denotes $P < 0.05$.

cases under observation during the first two weeks.

The explanation for this phenomenon can only be hypothetical

1 During the first stage of the disease many of these patients displayed only slight symptoms and did not therefore seek professional aid until they became worse. They were then remitted to hospital with some complication of the basic disease, this disturbing the more regular course of the basic disease itself

It was also found that a patient may indeed have sought professional advice t the ery beginning but then symptoms were so mild that hospitalization was not considered necessary Certain therapeutic measures were, of course, taken in such instances, but observation of the patient was of necessity less frequent and the therapeutic measures less effective than would have been possible after hospital nalyses. The primary test in mind in this connection is the determination of the antibiotic sensitivity of bacteria causing secondary infection. When this has not been done complications are liable to fog the picture of the disease to a greater extent than otherwise and thus deflect its course a great deal from that of the mean curve. This is clearly seen when the patient, because of the state of complication finally reaches the hospital

3 (In analogy with 1 and 2) Patients admitted to hospital at an early stage exhibit a more uniform course and a more uniform diagram than late intakes, since it is possible during the daily observations there to note the appearance of symptoms of complication and thereby be in a position to treat such complication by suitable therapy at the earliest opportunity The quantitative mean of atypical cells and lymphocytes will in this manner be lower than if such observation had not been carried out.

c. Interrelationship between Normal Blood Cells and Atypical Cells with Regard to Sex, Age and the Serology

The views just expressed concerning Tables 7 & 8 occasion the following queries and statistical analysis

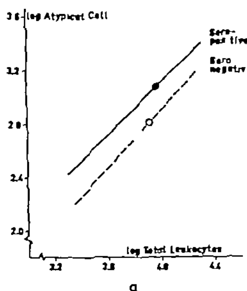
Is there any real difference between the groups adults/children, males/females and sero-positive/sero-negative cases with regard to the quantity of atypical cells? What is put in question here is firstly whether there is any difference between the respective groups mentioned with regard to the numbers of leukocytes, and secondly whether there is any correlation between the number of total leukocytes and the number of atypical cells. For if it should be found that there is a difference in the numbers of total leukocytes between the groups and if it should likewise be found that there is correlation between these numbers and the numbers of typical cells, then account must be taken of this correlation in order to justify an answer to the question as to whether difference between the particular groups regarding the quantity of atypical cells is in fact present or not.

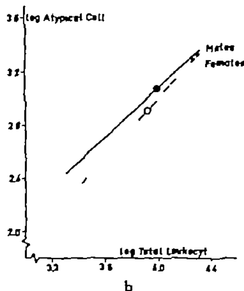
Table 10 presents the result of the significance analysis of the differences between the groups in question with regard to the number of total leukocytes. This was done by analysis of variance. It will appear from the table that a difference was found regarding the number of total leukocytes when the patient material was divided into males and females.

This result naturally led to correlation analysis of the groups (Table 11) This second analysis was carried out by employing the method of least squares on the number of total leukocytes (independent) and the number of atypical cells (dependent) It will appear from the table that there is a highly significant (Column VIII) correlation (0 415 Column VII) in this total material. Thus the number of atypical cells increases *on the average* by 1 073 (log) for a unit increase in the number of total leukocytes (Column VI) The correlation is also significant in all of the groups (Column VIII) The increase in the number of atypical cells per unit increase in the number of total leukocytes varies between 0.880 and 1 205 (Column VI)

The difference as to the atypical cells in the various groupings of the material of patients *without* regard to the regression (Column III) may be looked on as a gross difference consisting of three components 1) difference as a result of different numbers of total leukocytes, 2) real difference in the number of atypical cells and 3) difference due to chance effects.

If such correction is made, a net difference consisting of two components is obtained 1) a true difference in atypical cells and 2) a chance difference. This was done by covariance analysis, the result of which is presented in Table 11 Columns IX—XI. Column IX gives the significance of the gross difference,





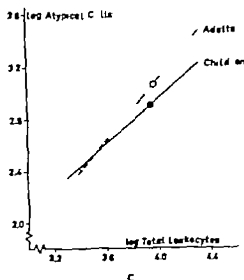


Fig. 4 —c. Demonstration of the Regression of log Atypical Cells on log Number of Total Leukocytes in Sero-Positive Cases, Sero-Negative Cases, Males, Females, Children and Adults.

Column X that of the net difference after correction with regard to the number of total leukocytes, and Column XI indicates whether the slope of the line of regression is significantly different for the various pairs of sub-groups. It will be seen that the gross difference is significant for males as opposed to females and for sero-positive cases as opposed to sero-negative. Significance as regards the gross difference is, however of secondary importance since the dependence on the number of total leukocytes still remains. The significance in the gross difference between males and females is effaced after regard is paid to the difference in total leuko

TABLE 12. *Result of correlation analysis between lymphocytes and atypical cells in the entire special material and its various groups. Log values*

	No. of cases	Total lymphocytes (mean)	Atypical cells			Coefficient of regression	Coefficient of correlation	t
			Mean and standard error	Standard deviation	Corrected standard deviation			Evaluating the significance of the correlation
Children	69	3.61	2.91 ± 0.06	0.49	0.49	0.206	0.159	1.14
Adults	91	3.61	3.07 ± 0.06	0.55	0.53	−0.427	−0.209	2.05
Males	81	3.64	3.08 ± 0.06	0.54	0.52	−0.505	−0.236	2.56
Females	79	3.58	2.91 ± 0.06	0.52	0.52	0.167	0.104	0.92
Sero-positive cases	112	3.64	3.08 ± 0.05	0.49	0.49	−0.229	−0.131	1.39
Sero-negative cases.	48	3.53	2.81 ± 0.08	0.58	0.59	−0.026	−0.015	0.10
Total	160	3.61	3.00 ± 0.04	0.53	0.53	−0.093	−0.032	0.66

Non-significant.
Denotes $P < 0.05$.

cytes (Column X) but remains between sero-positive and sero-negative cases thus as a significant net difference *howing that serological diagnosis — regardless of the number of total leukocytes — seems to b of importance as to the number of atypical cells*

In no instance did the line of regression show any difference in slope between the respective groups.

Figs. 4 a—c illustrate the result.

Analysis of significance carried out by means of analysis of variance (Table 10) was nowhere able to show any difference with regard to the number of lympho-cyt when the material was grouped into children adults, males females or sero-positive sero-negative cases.

From the clinical point of view the question as to whether there might be a correlation between atypical cells and lymphocytes is of interest. Correlation analysis with regard to the number of lymphocytes (independent) and the number of atypical cells (dependent) as carried out by means of the method of least squares (Table 12) showed that the total material exhibited no correlation. On division of the material in various ways (see the table) a significant corre lation was obtained only in the groups adults and males. In both these instances the correlation was negative i.e. an increased number of atypical cells was followed by a decreased number of lymphocytes.

CHAPTER 5

Concurrent Clinical Symptoms and Atypical Cells

It was considered to be of particular interest to investigate whether the oc curence of a number of concurrent clinical symptoms might not be the expression of a more severe form of the disease. For this reason, certain prominent symptoms present in common with the disease were investigated in relation to the occurence of atypical cells.

a. Palpable spleen compared with non palpable spleen

Analysis of variance showed that there was no significant difference between palpable and non-palpable spleen as regards the absolute number of atypical cells. The calculation is based on the cases in the special material 84 with palpable spleen and 76 with non-palpable spleen.

b Lymphoma at the pulmonary hilus and palpable spleen (Table 13)

After the summation of cases with and without secondary infection, it was found that there were 41 sero-positive cases without pulmonary hilus lymphoma or palpable spleen, whereas there were only 7 patients with both these symptoms. Atypical cells occurred to a 34 % greater extent in the latter There was only one patient among the sero-negative cases with both these symptoms as opposed to 20 without either of them.

Taking the sero-positive and sero-negative cases together there were 8 cases with both symptoms as opposed to 61 without either and these 8 cases ex hibited atypical lymphocytes to a 45 % greater extent than the 61 cases without symptoms.

When comparing the 8 cases with both symptoms with all remaining cases (135) — among which only one or none of these symptoms were present — analysis of variance showed that the difference as to the number of atypical cells was not significant, however

In order to investigate the simpler question as to whether cases with hilus lymphoma are more often accompanied by palpable spleen than cases without observable hilus lymphoma, χ^2 analysis was performed. This was found to be negative among both sero-positive and sero-negative cases as well as in the combined material. The material thus shows no parallelism between the presence of pulmonary hilus lymphoma and palpa ble spleen. Both symptoms together did not occur significantly more often among sero-positive patients than among sero-negative (the Fisher absolute probability test was negative)

Abnormal Cereb al Spinal Fluid (CSF) as judged by th occurren e f cells and palpable spleen (Tabl 14)

The CSF groupings were set up according to the same principles as those in Chapter 3

Here it was found as regards the sero-positive cases that patients having neither abnormal CSFs nor palpable spleens ex hibited a greater number of atypical cells than those with both these symptoms. This situation is reversed as regards the

TABLE 12. *Result of correlation analysis between lymphocytes and atypical cells in the entire special material and its various groups Log values*

	No. of cases	Total lymphocytes (mean)	Atypical cells: Mean and standard error	Atypical cells: Standard deviation	Atypical cells: Corrected standard deviation	Coefficient of regression	Coefficient of correlation	t Evaluating the significance of the correlation
Children	68	3 61	2.91±0.06	0.49	0 49	0 206	0.139	1 14
Adults	92	3.61	3.07±0.06	0.55	0.53	−0.427	−0.209	2.03
Males	81	3.64	3.08±0.06	0.54	0.52	−0.505	−0.256	2.36
Females	79	3.58	2.91±0.06	0.52	0.52	0.167	0.104	0.92
Sero-positive cases.	112	3 64	3.08±0.05	0.49	0.49	−0.229	−0.131	1.39
Sero-negative cases.	48	3.53	2.81±0.08	0.58	0.59	−0.026	−0.015	0.10
Total	160	3.61	3.00±0.04	0.53	0.53	−0.093	−0.032	0.66

Non-significant.
Denotes $P < 0.05$.

cytes (Column X) but remains between sero-positive and sero-negative cases, thus as a significant net difference *showing that serological diagnosis — regardless of the number of total leukocytes — seems to be of importance as to the number of atypical cells*

In no instance did the line of regression show any difference in slope between the respective groups.

Figs. 4 a—c illustrate the result.

Analysis of significance carried out by means of analysis of variance (Table 10) was nowhere able to show any difference with regard to the number of *lymphocytes* when the material was grouped into children/adults, males/females or sero-positive/sero-negative cases.

From the clinical point of view the question as to whether there might be a correlation between atypical cells and lymphocytes is of interest. Correlation analysis with regard to the number of lymphocytes (independent) and the number of atypical cells (dependent) as carried out by means of the method of least squares (Table 12) showed that the total material exhibited no correlation. On division of the material in various ways (see the table) a significant correlation was obtained only in the groups adults and males. In both these instances the correlation was negative, i.e. an increased number of atypical cells was followed by a decreased number of *lymphocytes*.

TABLE 14 *Cerebro-spinal fluid and palpable spleen in relation to the occurrence of atypical cells*

CSF	Spleen		PB positive		PB negative		All cases	
			No.	Atypical cells, mean value	No.	Atypical cells, mean value	No.	Atypical cells, mean value
0	0		17	1,313	9	393	26	863
0	1		23	1,363	8	966	31	1,247
		Total	40	1,341	17	600	57	1,055
1	0		13	1 014	6	481	19	801
1	1		16	1 182	5	611	21	1,010
		Total	29	1 104	11	536	40	905
2	0		9	1,593	4	417	13	1,053
2	1		9	860	7	1,100	16	958
		Total	18	1 170	11	773	29	1,000
0	0							
1	0							
2	0							
		Total	39	1,260	19	424	58	882
0	1							
1	1							
2	1							
		Total	48	1,192	20	902	68	1 100

CSF
- 0 = normal
- 1 = pathological ($\geq$ 5 cells/mm³)
- 2 = doubtful border group (3–4 cells/mm³)

Spleen
- 0 = normal
- 1 = palpable.

sero-negative cases as well as of the material as a whole.

Taking the CSF by itself, it was found that cases with normal CSFs had a greater number of atypical cells than cases with abnormal CSFs regardless of the EEG result. This was also true for the sero-positive and sero-negative groups as well as for the material as a whole. Taking the EEG alone the total number of cases with normal EEGs had a somewhat greater number of atypical cells than the total number of cases with abnormal EEGs. This was also true of sero-positive cases as well as sero-negative and of the material as a whole.

It can therefore be said that there is no positive correlation on the one hand between the occurrence of abnormal EEGs and/or abnormal CSFs and on the other hand between the occurence of an increased number of atypical cells.

The table further illustrates the interesting observation that patients may quite often have an abnormal CSF in combination with a normal EEG or vice versa,

TABLE 15. *Electroencephalogram and cerebro-spinal fluid in relation to the occurrence of atypical cells*

EEG	CSF	PB positive		PB negative		All cases	
		No.	Atypical cells, mean value	No.	Atypical cells, mean value	No.	Atypical cells, mean value
0	0	20	1,357	11	538	31	977
0	1	15	1 134	6	646	21	966
0	2	9	1 406	9	924	18	1 159
	Total	44	1,285	26	677	70	1 015
1	0	12	1 702	2	631	14	1 477
1	1	13	1,060	3	249	16	908
1	2	4	832	2	347	6	621
	Total	29	1,247	7	357	36	978
0	0						
1	0						
	Total	32	1 477	13	551	45	1 111
0	1						
1	1						
	Total	28	1,099	9	470	37	894
0	2						
1	2						
	Total	13	1 197	11	773	24	979

EEG { 0 = normal. 1 = pathological.

CSF { 0 = normal. 1 = pathological ($\geq$ 5 cells/mm³). 2 = doubtful border group (3—4 cells/mm³)

i.e. a normal CSF in combination with an abnormal EEG χ^2 analysis of the sero-positive cases as well as of the material as a whole and the Fisher testing of the sero-negative cases proved to be negative, i. e. there was no positive correlation between an increasing frequency of the one symptom with an increasing frequency of the other

Neither abnormal CSFs nor abnormal EEGs were found to occur more often among sero-positive patients than among sero-negative (negative χ^2 analysis)

e Cases with palpable cervical, axillary and inguinal lymph glands in combination with roentgenologically diagnosed lymphom at the pulmonary hilus

Pulmonary X ray examination was not carried out in 17 cases. Of the remaining material it was found that 9 cases had all the above mentioned lymph glands affected, 7 of them sero-positive and 2 sero-negative whereas the rest — having at the most only three of the gland sites affected — comprised 134 cases, 91 of them sero-positive and 43 sero-negative.

Analysis of variance showed that the difference in the quantity of atypical cells between the 9 cases with all symptoms present and the remaining 134 cases was significant (F = 5.06*)

	No. of cases	Atypical cells, Mean Value
All gland sites affected	9	2 222
Others	134	869

Result

The patients with all lymph-gland sites affected showed a greater number, statistically significant, of atypical cells than others. In the total material with the double symptoms of lymphoma at the pulmonary hilus and palpable spleen, there was found to be no significant difference in the number of atypical cells between patients with both these symptoms and other patients.

The total number of cases with abnormal CSFs (partly in Table 14 and partly in Table 15) showed a lower number of atypical cells than remaining groups in the respective tables, this being true for both sero-positive as well as sero-negative cases.

The total number of cases with abnormal EEGs in Table 15 showed a lower number of atypical cells than the cases with normal EEGs among the sero-positive cases as among the sero-negative even though the difference among the sero-positive was small

The total number of sero-positive cases with palpable spleen exhibited a somewhat greater number of atypical cells than cases without palpable spleen as shown in T ble 13, i.e. in combination with lymphoma at the pulmonary hilus or not, but not according to Table 14 i.e in combination with an abnormal CSF or not.

The total number of sero-positive cases with lymphoma at the pulmonary hilus (Table 13) exhibited a far greater number of atypical cells than corresponding cases without hilus lymphoma. This situation is reversed among sero-negative cases, but not to such a great extent.

When the number of atypical cells was excluded from the analysis it was found that the occurrence of one symptom did not give rise to an increased frequency of another symptom, at any rate as based on the studies reported on here on the paired symptoms lymphoma at the pulmonary hilus palpable spleen, abnormal CSF/palpable spleen and abnormal CSF/abnormal EEG

The serum reaction was found to have no regular influence on the frequency of the symptoms reported on here.

Discussion

It seems as if the appearance of an abnormal symptom is in no way regularly followed by an increased occurrence of other abnormal symptoms, even though an increased occurrence of atypical cells is included among the symptoms. This strengthens the earlier conception of the disease as being protean. The degree of severity of the disease can only to some extent be measured by the number of symptoms. It even seems improbable that the number of typical cells might be a yardstick for the degree of severity It is possible in this connection that the appearance of special typical cell forms among the white blood corpuscles might be a more correct means of measurement

It is possible that the occurrence of atypical cells with an appearance like that of

Downey's Type III could be an expression for a more severe form of the disease. Such division according to histological principles has not been undertaken in this work, however

One exception to this line of reasoning is constituted by the cases with all the four above-mentioned lymph-gland regions affected. These display a significantly greater value for the quantity of atypical cells. This will have to be interpreted as an expression that these patients suffer a greater lymphatic reaction than others.

CHAPTER 6

Examination of Patient Contacts

As far as was possible, those having had contact with the patients were examined. The individuals examined in this way were for the most part members of the patients families or close friends. The examination was performed in the out patient department of the hospital or in a few instances, by visits to the home. It was invariably performed by the author and constituted anamnesis, inspection of the general state of health of the individual, the throat, the various superficial lymph-gland regions and palpation of the abdomen with special regard to the possible occurrence of palpable spleen and/or liver. A blood sample was also taken at this examination and was investigated in the same manner as all other blood samples in this study by the same personnel. For practical reasons these samples could not be taken in the fasting state.

The method of assembling the contact material was that a list was made of patients who had contacts, and that a list was also made of the contacts themselves. In the instances in which there were more than one contact, a single contact was chosen at random. 14 patients had 2 contacts, one had 3 and one had 5.

In the one instance in which there was a mutual contact to more than one patient, the patient was chosen at random.

When this reduction of the material was completed, 38 contacts remained. These contacts thus correspond to one patient alone and vice versa. The age and sex distribution among the contacts will appear from Table 16. The ages of the boys and the girls are limited up to and including the age of 14 as in the clinical material.

Table 17 illustrates the findings of pathological clinical symptoms in the contact material. Among the individuals displaying some pathological clinical symptom in this material, there was a relative frequency of atypical cells within the frequency range 4—7.5 % in 4 cases as to the remainder the frequency was within the range 0—3.5 %. Thus no contact with any clinical pathological symptom displayed any surprisingly great relative frequency of atypical cells. The frequencies in the table for pathological symptoms are as will be seen, low — with the exception of upper respiratory tract infection (subjective information given by the individuals themselves) and pharyngitis (objectively diagnosed by the author) in which instances they are moderate. It should be noted, however that "subjective upper respiratory tract infection is only seldom accompanied by "objective" pharyngitis — in fact in only three cases. Generally speaking, the distribution of symptoms among these contacts is very scattered, and only one case, an adult male exhibited 4 symptoms upper respiratory tract infection, palpable spleen and liver and pharyngitis. Nevertheless, his relative frequency of typical cells belonged to the lowest frequency group of 0—3.5 %

All of these contacts with pathological symptoms have had contact with patients

TABLE 16 *Age and sex distribution among contacts*

Male adults.	10
Male children	2
Female dults.	25
Female children	1

with a relative frequency of more than 9 ‹ atypical cells.

Below is a report on the spread of the disease in a few cases of interest. As regards the blood tests the sampling oc casion chosen in these instances was that which showed the greatest frequency of atypical cells in the primary patient. Similarly the greatest occurring titre in the Paul-Bunnell test was selected.

Record No 4166/56 B. S man aged 20. Onset *8/10 temperature of up to 39 4* C, sore throat and enlarged cervical lymph glands. Number of total leukocytes on 22nd day of disease 16 700 per mm³ by differential count this was found to correspond to 31.5 % lymphocytes and 39 % atypical cells — which in absolute numbers amounts to 6,513 atypical cells per mm Positive Paul-Bunnell test, th titr being 40.

Re ord N 4314/56 B. A., man aged 22, the brother of the previous patient. Met the brother when th brother fell ill and complet ed his military service in another district without coming into further contact with his brother Onset 25/10 sore throat and temperature of up to 39 7 C. The number of total leukocytes was 12,200 per mm³ on the 8th day of disease; by differential count there were found to be 9.5 % lymphocytes and 46 5 % atypical cells — which in absolut numbers amounts to 673 typical cells per mm³ Positive Paul-Bunnell test, titre 40.

Contact t th se tw patient

B G the mother f th patient 52 years f g N subjective or objective pathological symptoms. Th umber of total leukocytes on examination on 10/11 was 5,800 per mm³ by differential ount there wer found t be 41 5 % lymphocytes and 0 5 % typical ells which in absolut umbers amounts to 29 typic l ell per mm

B M the sister of the patients 16 years of age She had head cold in the period around 8/10 otherwise no subjective or objective pathological symptoms. The number of total leukocytes was found to be 8,100 per mm at examination on 12/11 by differential count there wer found to be 44 % lymphocytes and 0 % atypical cells at examination on 12/11.

Thus in this family both brothers fell ill whereas the mother and sister appear to have escaped the disease. The incuba tion period for the elder brother was 17 days.

Record N 3295/56 R. A., man aged 18. *Onset 9/8 fatigue*, headache, a temperature of more than 38° C and throat trouble. The number of total leukocytes was 15,900 per mm³ on the 18th day of disease; by differential *count this was found t* be 33.5 % lymphocytes and 49 % atypical cells — which in bsolute numbers amounts t 7 791 atypical cells per mm³ Positive Paul Bunnell test, titre 40 The patient lodged with th follow ing family

Record No 3770/56 J S., boy aged 14 Onset 25/9 temperature of more than 39 C, tired, apathetic, sore throat, visibl swelling of the neck. The number of total leukocytes was found to be 7,300 per mm³ on the 8th day of disease· by differential count this was found to correspond to 60 % lymphocytes and 26 % typical cells — which in absolute numbers amounts to 1,898 typical cells per mm³ Positive Paul-Bunnell test, titre 40

Contacts t these two patient

J A., 42 years old mother J 3770/56 Examined on 3/10. N subjective or objective pathological symptoms. The number of total leukocytes was 8,100 per mm³ by differential count this corresponded to 41 % lymphocytes and 1.5 % atypical cells.

J S 52 y ars old father J 3770/56 Examined on 6/10. N subjective pathological symptoms. Objectively there was only a slight reddening of th throat, otherwise nothing pathological. Th number of total leukocytes amounted t 5 400 per mm³· by differential count this corresponded to 34.5 % lymphocytes and 1 5 % typical cells.

TABLE 17 *Contacts with various clinical symptoms*

Patient involved	Contact		Symptoms among contacts				
			Upper resp. tract inf. (subjective)	Abnormal lymph glands	Palpable spleen	Palpable liver	Pharyngitis
1573/56	K. M.	F		+			+
684	D. G	F		+	+		−
3784	E. G.	Ma	+				+
2714	D. K.	F	+				+
4897	Ö. H.	Mc	+	+			
2724	B. H.	Ma					−
4532	R. T	F			+		−
4472	F. O	Ma	+		+	+	+
4453	M. A.	F	+				
4860	J. B	F	+				
4514	B. M.	F	+				
2987	B. K.	Ma		+			−
2638	R. G.	Ma					+
518	R. K.	F	+				
		Ma	2	1	1	1	3
		M	1	1	0	0	0
		F	5	1	1	0	3
		F	0	1	1	0	1

Ma = Male adults. Mc = Male children. F = Female adults. F = Female children.

J. B. 17 years old, sister of 3770/56. Sore throat, moderate cold, sleeplness and temperature of up to 39 C for 1—2 days around 25/9. Examined on 7/10. Objective observation: 1—2 pea-sized, soft lymphomata on the left side of the neck. Number of total leukocytes was 5 400 per mm^3; by differential count this corresponded to 36.5 % lymphocytes and 0 % atypical cells.

As a comparison it may be mentioned that the brother (Record No. 3770/56) at examination on 11/10 had 4,300 total leukocytes per mm^3 of which 59,5 % were lymphocytes and 19 % atypical cells, and on 18/10 4,900 total leukocytes per mm of which 52.5 % were found to be lymphocytes and 14.5 % atypical cells.

Thus in this group of individuals two of them fell ill with clear symptoms of the disease whereas the third (J.B.) had subjective symptoms at the same time as the later definite case but did not exhibit any blood changes at an examination which took place on 15th day after onset of disease. On the other hand, the brother who fell ill at the same time, still showed clear signs of blood changes 11 days after sampling of the sister. The adults displayed no symptoms whatsoever.

At times there may be propagation of the disease without the appearance of any outward signs.

Record No. 4876/56. L. A., girl aged 14. Onset 18/11 with sore throat, headache and temperature of up to 38.8 C. Number of total leukocytes on 15th day of disease was 11 400 per mm^3; by differential count this corresponded to 35.5 % lymphocytes and 36.5 % atypical cells — which in absolute numbers amounts to 4,047 atypical cells per mm. Positive Paul-Bunnell test, titre 320.

C ... CHART ... C

L. J ... ther ... 4 ... years of age ... an ... ard ... jective signs of ... exami- ... by differ ... ded to 0.5 % ... pical ... lls — ... mount to ...

... toxis occurred ... he following

... man aged 32, ... nlarged lymph ... mperature of up ... al leukocytes on ... 0 per mm³, by ... nd to corre ... s and 38.5 % ... solute numbers ... ells per mm³ ... 320

... previous patient 32 ... y nothing pathologi- ... as a slight reddening ... soft palate and rear wall ... The number of total leuko- ... examination on 7/6 was 6,000 per mm³. Differential count showed this to be ... 60 % lymphocytes and 6 % atypical cells — which in absolute numbers amounts to 3,600 lymphocytes and 360 atypical ... per mm

The mother of the patient showed no ... of the disease at all, however

F. E. mother of 2721/56, 66 years of age. Subjectively and objectively healthy. At examination on 30/6 the number of total leukocytes was 6,700 per mm³, by differential count this was found to correspond to 34 % lymphocytes and 3 % atypical cells — which in absolute numbers amounts to 201 atypical cells per mm

Record No. 4148/55 D. J., girl aged 12. Onset 6/11 with a temperature of up to 39 C, sore throat and headache. Palpable spleen. Number of total leukocytes on 8th day of disease was 4,_00 per mm by differential count this was found to correspond to 62 % lymphocytes and 8 % atypical cells — which in absolute numbers amounts to 2,666 lymphocytes and 344 atypical cells per mm³. Positive Paul-Bunnell test, titre 40

Record No. 681/56 D. K., girl aged 11, previous patient's sister. Onset 26/1 with sore throat and rise in temperature to 39.4 C. Palpable spleen. Number of total leukocytes on 19th day of disease was 7,100 per mm³, by differential count this was found to be equivalent to 47 % lymphocytes and 26.5 % atypical cells — which in absolute numbers amounts to 1,882 atypical cells per mm³. Positive Paul-Bunnell test, titre 320

Record No. 878/56 D. G., girl aged 10, previous patient's sister. Onset 8/12 1955 with pyrexia, sore throat and coating on the tonsils, was under medical supervision in the home, given penicillin, and recovered after 8 days' treatment, but was tired afterwards. Fell ill again on 6/2 1956 with swelling of the throat, dizziness and pyrexia. Spleen palpable, liver palpable. Number of total leukocytes on 21st day of illness (reckoned from 6/2 1956) was 6,400 per mm³, by differential count this was found to be equivalent to 38 % lymphocytes and 10 % atypical cells — which in absolute numbers amounts to 640 atypical cells per mm³. Positive Paul-Bunnell test, titre 40

Record No. 879/56 D. M., girl aged 5, the previous patient's sister. Onset 8/2 with temperature of 38° C, headache and sore throat. Spleen not palpable. Total leukocytes on 24th day of disease were 5,100 per mm³, by differential count this was found to correspond to 41.5 % lymphocytes and 8 % atypical cells — which in absolute numbers amounts to 408 atypical cells per mm³. Paul-Bunnell test negative.

Contacts to these 4 patients

D. M. woman aged 33. The patients' mother. No subjective or objective pathological symptoms. Number of total leukocytes at examination on 10/2 1956 was 2,900 per mm³. By differential count there were found to be 41 % lymphocytes and 4 % atypical cells — which in absolute numbers amounted to 116 atypical cells per mm

D. G. man aged 39. The patients' father. No subjective or objective pathological symptoms. Number of total leukocytes at examination on 10/2 1956 was 5,100 per

mm³ On differential count this was found to correspond to 23 % lymphocytes and 6 % atypical cells — which in absolut numbers amounts to 306 atypical cells per mm³

Case 878 fell ill on 8/12 1955 with pyrexia, sore throat, white coating on the tonsils and superficial swelling of the throat. She was attended in the home by a medical officer given penicillin and recovered after 8 days but was afterwards tired. It cannot be definitely decided whether her falling ill on 6/2 1956 depended on a new infection or on an exacerbation of the disease she contracted on 8/12 1955. If it were an exacerbation of the earlier infection then she would be an interesting link between case 4148/55 and the others. If this is not the case, then the question arises as to whether the later group of illness (January—February) was due to direct incubation from the first case in the family or was a newly acquired incubation from outside. If the first possibility were correct then the incubation period would be about 82 days. Such a long period of incubation must be said to be less probable. The falling ill of case 878 on 8/12 would give an incubation period of 33 days at the most from the onset in case 4148 and 49 days at the most up to the onset in case 684

This problem will never be solved with certainty It should be noted that blood sampling of the first case to fall ill (4148/55) was made on 10/2 1956 and was then found to have a number of total leukocytes of 3,600 per mm³ 41 % of them being lymphocytes and 11 % atypical cells or in absolut numbers, 418 per mm³ — thus pointing to a high value.

In this family then, all children exhibited signs of disease whereas the parents were subjectively healthy and showed no objective signs of disease

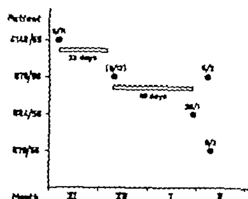


Fig. 5. Days of Onset of Disease for Four Members of One and the Same Family and Suggested Periods of Incubation for One of Them.

Their atypical cell frequencies were not clearly enhanced. It cannot with certainty be said whether the children contracted one or two infections.

The quantitative occurrence of atypical cells in the contacts has been related to that in the patients to whom the contacts belong. In so doing, the sampling occasion for these patients has been chosen as that being closest in time to the occasion when the contact in question was examined, this being referred to in Table 18 as closest value Furthermore a comparison has been made between the number of atypical cells in the contacts and the corresponding number in the adults of the healthy material (see Chapter 7) Comparison with the adults in the healthy material was made with regard being paid to the marked predominance of adults among the contacts — of the 38 contacts only 3 were children (Table 16) The patients have a greater number of atypical cells per mm³ than the contacts, whereas these in turn, have a greater number than that of the healthy material

TABLE 18. *Result of analysis of significance with regard to the number of atypical cells between patients contacts and healthy subjects*

Group	No.	Atypical cells: Mean and standard error log values	Geometric mean	Significances	F
Patients, closest value	38	3.09±0.10	1,230	Patients to healthy subjects	274.03
Contacts	38	2.17±0.06	148	Patients to contacts	100.63
Healthy subjects	37	1.78±0.05	60	Contacts to healthy subjects	20.37

Antilog of the previous column.

Note "healthy subjects" refers only t adults ≥ 16 years of ge.
contacts" refers t 3 individuals < 16 years of age and 35 individuals of the age of 16 or more
"patients" refers to 24 individuals < 16 years of age and 14 individuals of the age of 16 or more

The statistical analysis was performed by analysis of variance. The results are given in Table 18.

As will appear from the table there is a significant difference with regard to the number of atypical cells per mm³ between patients and their contacts (F = 100 63) and likewise a significant difference between contacts and healthy adults (F = 20 37) The difference is such that the presence of 3 children among the contacts does not influence the final result. The disease thus shows propagating ability from patients to contacts not only as regards outward symptoms but also haematologically as shown by an increased occurrence of atypical cells. This propagation is indeed f minor degree so that the difference between patients and contacts in this respect is great. *On the other hand there is a definite haematological difference in the number of atypical cells in the contacts is significantly greater than the number in healthy individuals This speaks in favour of there being very mild forms of infectious mononucleosis in the immediate vicinity of those suffering from it*

Summary

A fairly limited occurrence of subjective and objective superficial symptoms in the form of upper respiratory tract infection, abnormal lymph glands, palpable spleen, palpable liver and pharyngitis are reported on in 38 patient contacts.

Several contact chains are exemplified.

Haematologically a certain influence in the contacts is demonstrated with regard to an increased number of atypical cells statistical analysis does show that there is a significantly greater number of atypical cells in patients than in their contacts, but it also shows that there is a significantly greater number of atypical cells in these contacts than in healthy adul

CHAPTER 7

Study of Healthy Subjects

In order to inquire into the occurrence of atypical cells in healthy individuals, the following investigation was organized during the spring of 1956. It covered three different age groups of children (boys as well as girls) together with male and female adult individuals. To be certain that the investigation was carried out during the period freest from infection, the children were examined during their first week of school in the autumn term, that is to say just after their return to the city after the summer holidays. For this reason, the planning was organized during the spring of the same year so that the investigation could be made without delay at the appointed time. The project was carried out according to plan. The three age groups covered the years 7—9 10—1 and 13—16 each of them consisting of boys as well as girls. Three boys and three girls were more than 14 years of age. The examination of the adults was performed in an identical fashion, these individuals being selected from among students at the Royal Central Gymnastic Institute in Stockholm (instructor side) and examined immediately on their return to the institute after the summer vacation.

The examination constituted capillary blood sampling for the determination of the number of total leukocytes per mm followed by a differential count of them. The capillary blood sampling was performed by a competent laboratory nurse on the staff of the Hospital for Infectious Diseases. The number of total leukocytes was determined at the clinical-chemical laboratory of the hospital and the differential count was carried out by the one or the other of the two persons who performed the same work on the patient material and contacts in this study. Endeavours were made to obtain the samples in the fasting state, but for practical reasons this was not always possible to do.

Table 19 shows the result of the differential count in the various age and sex groups expressed as a mean percentage for the various cell types. Regarding the atypical cells the range is also given.

Table 20 illustrates the relative frequency of atypical cells expressed in percentages for several of the various groupings of the material.

Table 21 displays the means of atypical cells (in absolute numbers) per mm^3 in children and adults after statistical study by analysis of variance. It will appear from this table that the number is significantly greater among the children than among the adults. The same calculation for men and women in the group consisting of healthy adult individuals shows that there is no difference among them as regards sex grouping.

Table 22 shows the total leukocytes and atypical cells expressed as a mean log with division into sex and various age groups. This table demonstrates that no sex difference is to be found for these types of cells in any of these groupings.

The weighted mean of the total leukocytes among the children is log 3.75 whereas for the adults this is log 3.78. The number of atypical cells, on the

TABLE 19 *Healthy subjects: differential count of the white blood corpuscles (mean percentages). The material divided into sex and age groups*

	R leuko-cytes	S leuko-cytes	Eosino-phils	Baso-phils	Lympho-cytes	Mono-cytes	Plasma cells	Atypical cells	Range (atypical cells)
♂ 7—9 years	1.55	41.83	5.45	0.91	41.63	5.83	0.07	2.72	0.5–7.5
♀ 7—9 years	1.93	42.11	4.59	0.45	43.33	5.11	0.18	2.29	0–5.5
♂ 10—12 years	1.50	42.34	4.71	1.0	42.73	5.14	0.16	2.20	0–5.5
♀ 10—12 years	1.48	45.09	4.67	0.68	39.97	5.81	0.15	2.16	0–6.5
♂ 13—16 years	1.93	48.27	3.55	0.63	36.87	6.43	0.07	2.23	0–4.5
♀ 13—16 years	1.82	47.30	4.02	0.55	37.05	6.63	0.17	2.47	0–9
♂ Adults	3.57	56.16	1.81	0.59	31.17	5.28	0.03	1.38	0–4.5
♀ Adults	3.61	57.68	1.46	0.48	30.02	5.13	0.14	1.27	0–3

other hand, was greater among the children than among the adults (Table 23). Since there is thus a reverse situation as regards the quantity of total leukocytes and the quantity of atypical cells between children and adults, it cannot be expected that the difference between them as regards the atypical cells should be positively dependent on the quantity of total leukocytes.

The situation is illustrated in fig. 6 in which the broken lines join the quantitative means of the respective cell types among the children and the adults. As will be apparent these broken lines exhibit divergence. This divergence is an expression for there being a tendency that increasing values in the quantity of total leukocytes is accompanied by decreasing values in the quantity of atypical cells.

For this reason it was not found statistically necessary to perform correlation analysis regarding the number of total leukocytes and the number of atypical cells among the adults and the children as a whole.

The material of children was further analyzed statistically (Tables 24 and 25). Table 24 shows the result of an analysis of variance to illustrate the question of sex and age differences with regard to total lekocytes and lymphocytes. It will appear from the table that there is no difference as regards sex for either cell types. On the other hand there is a significant difference between the age groups for the total leukocytes ($F = 5.36$) as well as for the lymphocytes ($F = 10.55$).

Table 25 illustrates the result of correlation analysis with regard to the total leukocytes (independent) and the number of atypical cells (dependent) carried out by means of the method of least squares. In the entire material there is a highly significant ($t = 4.50$) correlation (coefficient of correlation $= 0.262$). In this way the number of atypical cells increases

TABLE 20 *Healthy subjects. Relative frequency of atypical cells (mean)*

Total material	2.1 %
Boys	2.4 %
Girls	2.3 %
Children	2.3 %
Adults	1.3 %
Males	2.2 %
Females	2.1 %

TABLE 21 *Healthy subjects: result of analysis of significance of differences with regard to the quantity of atypical cells in children and adults and in adult males and females*

Healthy subjects	Atypical cells			
	No.	Mean and standard error log values	Geometric mean	
Children	280	1.98 ± 0.03	96	Significant difference between children and adults. F = 9.65 **
Adults	57	1.78 ± 0.05	60	
Males, adult	29	1.79 ± 0.07	62	No difference found between the sexes.
Females, adult	28	1.76 ± 0.07	58	

Antilog of the previous column.
I this division of the material, the demarcating line was drawn between the age of 16 and 17

TABLE 22. *Healthy subjects. the number of total leukocytes and atypical cells in different sex and age groups Log values*

	Total leukocytes				Atypical cells			
	Males		Females		Males		Females	
	No.	Mean and standard error	No.	Mean and standard error	No.	Mean and standard error	No.	Mean and standard error
7—9 years	44	3.79 ± 0.02	38	3.78 ± 0.02	44	2.17 ± 0.04	38	2.03 ± 0.07
10—12 years	77	3.74 ± 0.01	61	3.73 ± 0.01	77	1.89 ± 0.07	61	1.98 ± 0.05
13—16 years	30	3.72 ± 0.02	30	3.74 ± 0.02	30	1.95 ± 0.09	30	1.97 ± 0.09
Total	151	3.75 ± 0.01	129	3.78 ± 0.01	151	1.98 ± 0.04	129	1.98 ± 0.04
Adults	29	3.77 ± 0.02	28	3.79 ± 0.02	29	1.79 ± 0.07	28	1.76 ± 0.07

on the ave age by log 1.061 for a unit increase in the number of total leukocytes. In the sub-groups there is similarly a significant correlation in both the two youngest ge groups but not in the oldest (see column t evaluating the significance of the correlation)

The column Mean and Standard Error presents the numbers of typical cells the various age groups expressed as logarithms. The differences between them were analyzed by covariance analysis, the result of which is given in the last three columns to the right the table F_1 shows that the gross differences are significant. Of greater importance however is F_2 which illustrates the net differences, i.e. after eliminating the dependence on the total leukocytes. F_2 was found to be non-significant. This means that no difference between the age groups with regard to the atypical cells seems to be present after the differences among the total leukocytes have been taken into consideration. F_3 is non-significant, indicat ng that there is no difference between the regression lines for the various sub-groups

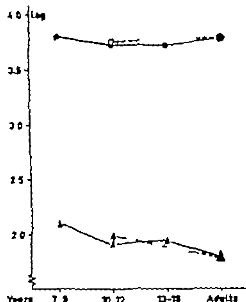


Fig. 6. Means of Total Leukocytes and Atypical Cells at Various Ages Illustrating Table 23.

The broken lines join the quantitative means of the respective cell types among the children and the adults.

● *Total leukocytes,*

▲ Atypical cells,

○ mean value for the children,

△ mean value for the children.

TABLE 23. *Healthy subjects, total leukocytes and atypical cells by age groups only. Means, log values*

	Total leukocytes	Atypical cells
Age 7—9 years	3.79	2.10
Age 10—12 years	3.74	1.93
Age 13—16 years	3.73	1.94
Total (all children)	3.75	1.98
Adults	3.78	1.78

Discussion

In this material of blood samples taken from healthy individuals there appear atypical cells within the range 0—9 % *expressed as a relative frequency.* The greatest frequency occurs in the child material; among the adults the range of relative frequency lies between 0 and 4.5 %. A *point of interest* however is the fact that the greatest relative frequency recorded, that of 9 %, was found in the oldest child age group. When the mean of the relative frequency figures among the children is placed in relation to the corresponding mean for the adults, a difference of 1 unit is obtained: 2.3 % against 1.3 (Table 20). The sexes display very similar figures in the two age groups.

Calculated in absolute numbers, it is *found that there is a significant difference* between the total child material and the adults (Table 21). No difference between the sexes was found.

In the child material there is a statistically significant difference regarding total leukocytes as well as lymphocytes between the various age groups, the greatest frequency for total leukocytes as well as lymphocytes being found in the youngest age group (Table 24). Regarding the atypical cells there is similarly a significant difference as far as the absolute numbers for the atypical cells alone are concerned (Table 25). But when the changes in the number of total leukocytes is taken into account so that a net difference as regards the atypical cells is calculated, the difference becomes non-significant, indicating that the statistical analysis, when account is taken of the differences in the number of total leukocytes, no longer demonstrates that there is any difference regarding the atypical cells, in other words indicating that the ratio between the total leukocytes and the atypical cells is constant.

The material has thus exhibited a greater occurrence of the number of total leukocytes and lymphocytes in younger children than in the older and the adults and that the similar observation of a

TABLE 24. *Healthy subjects: result of analysis of significance of the differences between boys and girls and the various age groups among the children irrespective of sex but with regard to the number of total leukocytes and lymphocytes. Log values*

	No. of cases	Total leukocytes: Mean and standard error	Total leukocytes: Standard deviation	Total leukocytes: F Evaluating the significance of the differences between means	Lymphocytes: Mean and standard error	Lymphocytes: Standard deviation	Lymphocytes: F Evaluating the significance of the differences between means
Boys	151	3.75±0.01	0.12	0.18	3.35±0.01	0.15	0.12
Girls	129	3.76±0.01	0.11		3.35±0.01	0.14	
Age 7—9 years	82	3.79±0.01	0.11		3.40±0.02	0.14	
Age 10—12 years	138	3.74±0.01	0.11	5.36*	3.35±0.01	0.14	10.55
Age 13—16 years	60	3.73±0.02	0.11		3.29±0.02	0.15	
Total	280	3.75±0.01	0.12		3.35±0.01	0.15	

Note. Within all age groups the difference between the sexes as to "total leukocytes" and "lymphocytes" were non-significant.

Non-significant. Denotes $P < 0.05$.

TABLE 25. *Healthy subjects: result of correlation analysis in combination with variance analysis between total leukocytes and atypical cells in the entire material and in the various age groups among the children. Log values*

Age group (years)	No. of cases	Total leukocytes (mean)	Atypical cells: Mean and standard error	Atypical cells: Standard deviation	Atypical cells: Corrected standard deviation	Coefficient of regression	Coefficient of correlation	t Evaluating the significance of the correlation	F	F	F
7—9	82	3.79	2.10±0.01	0.36	0.33	0.710	0.223	2.03			
10—12	138	3.74	1.93±0.04	0.51	0.49	1.325	0.296	3.61	3.76	*2.05	*2.47
13—16	60	3.73	1.91±0.08	0.47	0.4[illegible]	0.050	0.121	*0.92			
Total	280	3.73	1.98±0.03	0.47	0.4	1.061	0.262	4.50			

Evaluating the significance { in F of the difference between unstandardized means, in F of the difference between standardized means and in F of the difference between regression coefficients

Non-significant. Denotes $P < 0.05$

greater occurrence of atypical cells among the younger children *seems to depend on this greater occurrence of total leukocytes and not on an absolute greater occurrence of atypical cells*

On comparison with the material in this study which consists of patients suffering from infectious mononucleosis, it will be noticed that there is in fact a significant net difference between sero-positive and sero-negative cases regarding the atypical cells, the greater occurrence being found among the sero-positive. On the other hand no difference is to be found there when the patients are divided into the groups children/adults or males/females. Nor is there any net difference between the various age groups in the child material of *healthy* individuals with regard to the atypical cells. Taken as a whole, this may be looked on as further support for the opinion put forward in Chapter 3 and Chapter 4 that sero-positivity in infectious mononucleosis is an expression of a more advanced form of the disease manifesting itself haematologically in a greater occurrence of atypical cells.

Summary

The occurrence of atypical cells of the same type as met with in infectious mononucleosis is demonstrated in a material consisting of healthy individuals divided into age and sex groups. It is shown statistically by analysis of variance that there is a significantly greater occurrence of atypical cells among children than among adults. Comparison between the adults and the children as a whole demonstrates a reverse situation regarding the quantity of total leukocytes and the quantity of atypical cells in that the children exhibit a somewhat *lesser* number of total leukocytes but a *greater* number of atypical cells than the adults.

The material of children is divided into various age groups. The youngest age group displays the greatest absolute occurrence of total leukocytes as well as lymphocytes and atypical cells. There is no sex difference regarding any of these cell types when the entire number of boys is compared with the entire number of girls, and, as far as the total leukocytes and atypical cells are concerned, this is also found to be true for the various age groups. Analysis of significance demonstrates a significant difference between the age groups as regards the total leukocytes and lymphocytes. Correlation analysis in combination with covariance analysis shows that the difference between the age groups as regards the number of atypical cells is no longer statistically significant after account has been taken of the difference with regard to the number of total leukocytes.

GENERAL SUMMARY

An account is given of the symptomatology of infectious mononucleosis on the basis of findings from a patient material suffering from the disease. The haematological state is studied by statistical analysis with special reference to the quantitative occurrence of atypical cells in various groupings of the material. Also studied is the quantitative occurrence of atypical cells in contacts to the patients and in healthy individuals, and a statistical analysis of differences between patients, contacts and healthy individuals is made with regard to the quantity of atypical cells.

Chapter 1

This is a relatively short, summarizing account of earlier papers on infectious mononucleosis and closely related fields. An attempt is made to group later papers under the headings Serology, Pathological Anatomy, Biochemical Disturbances, Involvement of the Central Nervous System, Aetiology, Infectiosity and Differential Diagnosis. Some reports are given of atypical cells and their occurrence in different states of disease and in healthy individuals.

Chapter 2

The cytological character of the atypical cells is described. The various forms for the atypical cells classified are not only those according to Downey from 1923 but also other transitional forms such as the lymphocytoid/monocytoid of Glanzmann from 1930 and Lehndorff-Schwarz from 1932. The criteria employed in the study for the differentiation between normal and atypical cell in doubtful instances of close relationship to the lymphocyte are described. In this context the cell has been judged atypical if the cytoplasm was of definitely foamy character and/or displayed clear basophile enhancement.

Chapter 3

The material of patients consist of 238 cases of infectious mononucleosis diagnosed at the Hospital for Infectious Diseases, Stockholm, during the years 1955 and 1956. Diagnosis was made clinically and not serologically. Resulting from this is the division into sero-positive and sero-negative cases. The symptoms generally found in connection with the disease are primarily treated in the tables. Particular interest is paid to the 160 cases which underwent special haematological analysis (referred to as the "special material") cases which comprise the material for the statistical analysis of quantitative haematological states reported on in Chapter 4.

The "special material" is also divided in the tables into sero-positive/sero-negative cases, males/females and children/adults for the percentage distribution of the symptoms. These groupings give 112 sero-positive and 48 sero-negative cases, 81 males and 79 females, and 68 children and 92 adults. The analysis took certain consideration of whether a case had exhibited secondary infection or not.

Given separately is the greatest relative frequency of atypical cells (47.7 % of cases without secondary infection were found to have a relative frequency of

atypical cells of 15 % or more) The arithmetic means of the first and the greatest erythrocyte sedimentation rate test are also given (25 6 mm and 31.6 mm respectively)

From the simultaneous occurrence of palpable lymph glands in the cervical, axillary and inguinal regions, from the occurrence of membranous angina and palpable spleen, and from the duration of pyrexia, it is also shown that the frequency of these findings among the sero-negative cases is so great that it is not necessary from the clinical point of view to have obtained a positive serological reaction for the diagnosis of infectious mononucleosis. Serologically positive cases suffer more seriously from the disease, however

Chapter 4

Account is given of the quantitative occurrence of the various white blood cells in infectious mononucleosis, with special regard to atypical cells. The material is presented in its entirety and also in the groupings sero-positive/sero-negative cases, males/females and children/adults. The means by differential count display a marked relative lymphocytosis of approximately 50 % in all groupings. There is a great relative frequency of atypical cells with certain difference between the groups a greater frequency among sero-positive cases than among sero-negative among males than among females and among adults than among children. Rod-nucleated leukocytes display a frequency which is somewhat greater than normal. The tendency seems to be that the groups of the material which have given indication of a weaker lymphocytic reaction have a somewhat greater relative figure for rod-nucleated leukocytes, this being most apparent in the sero-negative group

The quantity of total leukocytes calculated in absolute numbers shows a geometric mean per mm³ which lies a little above the normal upper limit. The geometric means for lymphocytes and atypical cells show high values. The number of rod-nucleated and segmented leukocytes, on the other hand, does not seem to be definitely influenced. The same differences are reflected within the various groupings of the material as those demonstrated in relative form in the result of the differential count.

Certain graphic analysis was carried out with regard to the quantitative progression of the numbers of total leukocytes, R & S leukocytes, lymphocytes, atypical cells and monocytes in sero-positive and sero-negative cases without secondary infection, cases which were examined haematologically within the first fortnight after onset.

The curve for the atypical cells in the sero-positive cases exhibits a clear falling tendency whereas the curves for the other cells is flat and almost horizontal. The atypical cell curve for the sero-positive cases lies initially above the corresponding curve for the sero-negative cases up to the 9th day of disease.

Statistical analysis as regards the quantity of total leukocytes, lymphocytes and atypical cells was carried out between the groups children/adults, males/females and sero-positive/sero-negative cases. Analysis of significance demonstrates a difference in the number of total leukocytes between the patient groups males/females. With regard to the lymphocytes, there is no difference between the various groups of the patient material.

Correlation analysis shows that there is correlation between the number of atypical cells and total leukocytes in all

groups and in the material as a whole. Analysis of covariance with correction for variation in the number of total leukocytes demonstrates that there is a *significant difference with egard to the quantity of atypical ells betw en sero-positive and se o-negative cases the greater occurrence being am ng the sero-positive*

Concerning the relationship lymphocytes:atypical cells, correlation analysis shows that correlation between these two types of cells is present only in the groups adults and males. In both these instances the correlation is negative.

Chapter 5

An account is given of a number of prominent concomitant symptoms in relation to the quantity of atypical cells. A relationship between clinical findings and the frequency of atypical cells is found only with regard to the symptom complex palpable cervical, axillary and inguinal lymph glands in combination with lymphoma at the pulmonary hilus.

Chapt 6

38 contacts to the patients have been examined. Clinical symptoms were to be found only sporadically among them. Thus one individual displayed abnormal lymph glands and palpable spleen, another palpable spleen and palpable liver. Abnormal lymph glands were found in three other ontacts and palpable spleen in further instance. The more difficultly diagnosed symptoms of upper respiratory tract infection (subjective and pharyngitis were found in totals of 8 and 9 of the ontacts respectively.

Haematologically the contacts display a far lower number of atypical cells than the patients, but on the other hand, a significantly greater number than the healthy individuals reported on in Chapter In spite of the infrequent occurrence of clinical symptoms, *ther is thus a haematological reaction among contacts to patient who hav been diagnosed as suffering from nfectious mononu leosis which also peaks in favou of there being a spr ad of the inf ction in the mmediate vicinity of those suffering from it*

Chapter 7

An account is given of atypical cells in healthy individuals — adults and 3 groups of children. This material consists of 57 adults and 280 children. *It is d mon st ated by analysis of variance that there is a significantly gr ater occurrence of atypical cells among the hild en than am ng the adult* The greatest relative frequency (9 %) was found among the girls in the age group 13—16 years. A relative frequency of up to 4.5 % was observed among the adults.

In the 3 groups of children, the youngest (7—9 years) exhibits the greatest absolute number of both total leukocytes and lymphocytes as well as of atypical cells. Analysis of significance demonstrates a statistically significant difference among the total leukocytes and lymphocytes in the various age groups. *Correlation analysis n ombination with analysis of ovariance demonst ate that the dif feren betw en th age group among the hild en with gard to the number of atypical lls is not significant when ac ou t is taken of the differen in th n mber of total leukocyte*

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With special reference to the limitation of the maximal physical working capacity

By

TORE STRANDELL

ACCOMPANIES VOL. 175

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Contents

The present publication is based mainly on studies reported in the following papers

I STRANDELL, T Electrocardiographic findings at rest, during and after exercise in healthy old men compared with young men. Acta med. scand., *1 4* 479 1963

II. STRANDELL, T Heart rate, arterial lactate concentration and oxygen uptake during exercise in old men compared with young men Acta physiol scand., *60* 197 1964

III STRANDELL, T Heart volume and its relation to some anthropometric data in old men compared with young men. Acta med. scand. 1964 In print.

IV STRANDELL, T Total haemoglobin, blood volume and haemoglobin concentration at rest and circulatory adaptation during exercise in relation to some anthropometric data in old men compared with young men. Acta med. scand 1964 In print

V STRANDELL, T Heart rate and work load at maximal working intensity in old men. Acta med. scand. 1964 In print.

VI STRANDELL, T Mechanical systole at rest, during and after exercise in supine and sitting position in young and old men. Acta physiol. scand. 1964 In print.

VII GRANATH, A., JONSSON B and STRANDELL, T Circulation in healthy old men, studied by right heart catheterization at rest and during exercise in supine and sitting position. Acta med. scand. 1964 In print.

VIII GRANATH, A. and STRANDELL, T Relationships between cardiac output, stroke volume and intracardiac pressures at rest and during exercise in supine position and some anthropometric data in healthy old men. Acta med. scand. 1964 In print.

In the text these will be quoted as (I) and (II) etc.

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Introduction

It is a wellknown fact that the maximal physical working capacity decreases with rising age in adults, at least above 30 years of age. The maximal physical working capacity is dependent on most functions of the body including all those taking part in locomotion, but is generally most associated with circulatory respiratory muscular and metabolic functions. The decrease of the maximal physical working capacity with age may thus be attributed to changes in these functions and perhaps partly to psychological causes — namely less willingness for physical exhaustion in old subjects.

The present investigation concerns some aspects of the circulation at rest and during exercise in a group of apparently healthy old men. A complete evaluation of the changes in circulation in old age is not possible nor intended in this paper. The following questions have been studied:

1. In what main respects does the circulation at rest differ between old and young men?
2. What are the differences between old and young men in adaptation of the circulation during exercise?
3. Do differences in central circulation between old and young men explain the differences in maximal physical working capacity, i.e. is central circulation the limiting factor for the working capacity in old age?

CHAPTER I

Material and Methods

Material

The findings in the present report are mostly based on data from 126 healthy male volunteers aged 30—83 years. All of these subjects up to 59 years of age were randomly drawn from the healthy men in the 1954 Health Survey of the City of Stockholm (Frisk et al. 1959 Carlson 1960). Of the 39 subjects above 59 years of age 14 were drawn from this source, the others being volunteers from the Stockholm Labour Exchange (9 cases) from old age homes (9 cases, all living in their own flats and being ambulatory) and from gymnastic groups for the aged (7 cases). In one study (VI) 12 healthy medical students aged 20—25 years were used as controls for comparison with old men.

Before acceptance for the study the subjects passed a clinical examination described in previous report (I). The upper limit for arterial blood pressure in subjects above 60 years of age was 160/100 or 175/80 mm Hg (Master and Lasser 1961). Two subjects who did not fulfil the criteria for acceptance but were included in studies of the central circulation (VII VIII) were discussed in detail in paper VII. The further selection of subjects for the different studies was reported in each separate paper.

Methods and Procedure

Detailed description of methods and procedures were reported in the separate papers and are therefore only briefly summarized below.

Heart volume (ml) was determined by X-ray in prone position by the method of Larsson and Kjellberg (1948) with slight modifications introduced by Kjellberg et al. (1949 1951a).

Total haemoglobin (g) was determined by the alveolar carbon monoxide method (Sjöstrand 1948) with some modifications (Wiklander 1956, Linderholm and Sjöstrand 1956, Linderholm and Söderström 1957).

Blood volume (l) was determined from total haemoglobin and haemoglobin concentration of finger blood.

Static lung volumes (l BTPS) were determined in sitting position by the helium dilution method using a closed spirometer system (Meneely et al 1949 Holmgren 1954). Conventional symbols were used.

Ventilatory tests were performed with a spirometer as described by Bernstein et al. (1952). Maximal voluntary ventilation (MVV) was determined as well as forced vital capacity (FVC) including forced expiratory volume in one second ($FEV_{1.0}$) and $FEV_{1.0}$ expressed as a percentage of the vital capacity (FEV%).

Electrocardiograms were recorded by a direct-writing ink jet recorder (Mingograph 42, Elema Järnh., Stockholm) both at rest in the supine and standing positions, during exercise on a bicycle ergometer in sitting position, and in the supine position 1/2—1 minute after and 4 minutes after exercise. At rest leads I II, III, aVR, aVL, aVF CR_{1-6} and V_{1-6} were used, during exercise leads CH_{1-6} with the indifferent electrode in the forehead (Sjöstrand 1951 Holmgren and Strandell 1961). The findings concerning the QRS complexes, ST segments (ECG_{ST}) ventricular ectopic beats and supraventricular ectopic beats were independently graded into five classes, class one being normal and class five being regarded as abnormal. A horizontal or sagging ST segment depression $\geq$ 0.05 mV (0.5 mm) at rest during or after exercise was

regarded as abnormal, as were frequent ventricular or supraventricular ectopic beats and two or more in series or ectopic beats from more than one focus. The total assessment of the electrocardiogram (ECG_{tot}) was based on the most marked abnormality recorded. In some subjects the electrocardiogram was also recorded during and after supine exercise.

The exercise test consisted of stepwise increased work loads (Sjöstrand 1960) on an electrodynamically braked bicycle ergometer (Holmgren and Mattson 1954) in sitting position, starting at 300 kpm/min. This was increased every sixth minut by further 300 kpm/min until the subject was exhausted. The work load at maximal working intensity (W_{max}) was taken to be the heaviest load at which the subject worked for 6 minutes with an increment proportional to the completed period at the next higher load.

Heart rate was determined after 2, 4 and 6 minutes at each load and before the interruption of the test by electrocardiographic recording of at least 10 beats, generally 25. Maximal heart rate (HR_{max}) was taken to be the highest heart rate recorded during exercise. Respiratory rate was calculated after 3 minutes at each load. Using the approximately linear relationship between heart rate and work load the working intensities in kpm/min corresponding to heart rates of 170 and 130 beats/min (W_{170} and W_{130}, respectively) were obtained by intra- or extrapolation. These values were used as estimates of the oxygen pulse during exercise at these heart rates.

The increase in heart rate from the second to the sixth minute at the load, the steady state of the heart rate, was at 600 kpm/min denoted St_{600}. The 2—6 min heart rate increase at heart rate 130 (St_{130}) was determined by intra- or extrapolation like W_{130}.

This exercise test was also performed in supine position, with the same measurements. In some cases a test with combined arm and leg work was performed, in which the subjects cranked with the arms on a separate ergometer simultaneously with the leg work (V).

The arterial lactate concentration (mE/l) was determined from arterialised finger blood by the colorimetric method of Barker and Summerson (1941) as modified by Ström (1949). As the logarithm of the lactate concentration during this exercise test was approximately linearly related to heart rate, the log lactate concentration at heart rate 130 (Log $lact_{130}$) was estimated by intra- or extrapolation. The log lactate at 600 kpm/min was denoted Log $lact_{600}$. Log lactate at maximal working intensity (Log $lact_{max}$) was either measured or estimated by short extrapolation from submaximal up to maximal heart rates.

Oxygen uptake (l/min) was determined by the Douglas bag technique, expired air being collected between the fourth or fifth and the sixth minute at each load and immediately measured by water displacement at atmospheric pressure from one tank to another (Stern 1959) or through a dry gas meter. Gas analyses were performed according to the Haldane technique. For determination of the oxygen uptake at maximal working intensity (Vo_{2max}) expired air was collected during the last 30—60 seconds of work.

Mechanical systole (csec) of the left ventricle was measured as the time interval on the phonocardiogram between the first vibrations of the 1st and 2nd heart sounds recorded with the filters at nominal frequencies of 100 c/s or 400 c/s. The phonocardiograms were recorded over the 4th left intercostal space with a direct-writing ink jet recorder (Mingograph 42 + 42 B Elema Jiirnh., Stockholm) at a paper speed of 100 mm/sec.

Cardiac output was measured during right heart catheterization according to the direct Fick method. During exercise expired air was generally collected for —3 minutes between the third and sixth minute at each load. Blood samples were simultaneously drawn from the pulmonary and brachial arteries and the oxygen content was calculated after spectrophotometric determina-

tion of haemoglobin concentration and oxygen saturation (Drabkin 1950 Holmgren and Pernow 1959)

Pr ssur e ording were performed with Swema Elema strain-gauge manometers or differential transformer transducers connected to amplif er units and recorded on an Elema light beam oscillograph (Mingograph) The arterial pressures wer transmitted through percutaneously introduced polyethylene or teflon catheters (Seldinger 1953) the other pressures generally through doubl lumen catheters. Mean pressures were obtained by electric damping

The reference point for zero pressure in the supine position was taken to be the mid-thoracic level of the sagittal chest diameter measured at the insertion of the fourth rib at the sternum. In the sitting position the insertion of the fourth rib at the sternum was taken as the reference point for zero pressure.

The pulmonary esistanc index was calculated as the differenc between the mean pressure in the pulmonary artery and the mean pulmonary capillary enous (PCV) pressur divided by cardiac output, and the systemic esistanc index as the mean pressur in the brachial artery divided by cardiac output

The d gr f phy ical activity at examina ion and earlier in life was graded ac ording t history in three classes, class one denoting no egula physical training, class tw moderat degree of training and class three high degree f training such as hard bicy ling every day cross-country running or hard ork g in the building trade

Stat t al al lat as ere mainly performed ac ording to Snedecor (1959) Differences between egression lines wer tested ac ording t Hald (1960) The following probabilit P les ls of significance wer used P 0.001*** highly significant 0.001 $P < 0.01$** signific nt and 0.01 $P < 0.05$ probabl ignificant Multiple egres an l ses performed ord- ing method of least squares.

T p m l p w d started after hr l exa ination h an exercise test in sitting position in the morning after a light morning meal, usually followed by determination of total haemoglobin, blood volume, heart olume and spirometric investigation. When repeated work tests were performed, they were usually carried out once or twice a week. After these determinations right heart catheterization was done in some ambulatory subjects without pre medication. All subjects were thoroughly informed about the procedures and were made familiar with the different tests.

Discussion

All transversal studies of different age groups are attended by problems of selection which nder conditions concerning the longitudinal changes with ag diff cult or impossible. The present material is highly selected in that the older age groups increasingly epresent a positive selection of individuals who, despite their age, are clinically healthy The differences between old and young men observed in this study may thus not necessarily be egarded as age changes *per se* but may also include othe differences between the old and young men studied. The effect of th selection was discussed in greater detail in paper I

It might be argued that the subjects above 60 years of age who were repeatedly tested and sometimes performed three t four maximal tests w thin two weeks were not in their normal circulatory state at the end of this period. In young sedentary subjects the same testing procedure should certainly ffect the heart at response during exercise Old subjects, however seem to change their circulatory adaptation during exercise less rapidly on training than young There were thus no differences of probabl significanc between W ro and W ax t the first test and the values obtained t subsequent tests in sitting position. The effect f training was furthermor studied in six of the old subjects by epeated maximal tests four times week for bout one month (Granath et al. 1962) The effect on the heart rat esponse during exercise was mod-

erate; t an oxygen uptake of 1.5 l/min the average decrease in heart rat was 10 beats/min and the maximal heart rate was not changed.

Some of the variables used in the statistical treatment were known not to be normally distributed, such as degree of physical activity assessments of the electrocardiographic findings and age. These variables were therefore generally used only as independent ariables in regression studies, but in a few instances they were used as dependent ariables or in correlation studies. The statistical significance of these relationships might therefore be less exact than the given values of probability But as it was repeatedly observed in control studies that the significance of the incorrect correlation coefficients and th correct regression coefficients was just the same, the de iation from normal distribution of these variables would not seem to be of such magnitude as t introduce errors in the statistical calculations.

CHAPTER II

Circulation at Rest

With advancing age marked and progressive structural changes occur in the cardiovascular system, mainly associated with increase in thickness and rigidity of the walls of the larger arteries and veins (Bourne et al. 1961). There is also a progressive increase with age of coronary artery sclerosis (Lober 1953) and changes take place within the heart walls with degenerative and sclerotic lesions in the endocardium and increase of the elastic tissue in the myocardium and epicardium (Bourne et al. 1961). These structural changes will not be discussed here but it should be noted that it is impossible to distinguish clearly between degenerative changes and normal physiological alteration with age.

Previous Investigations

Marked changes in the electrocardiogram at rest occur with increasing age in adults. When normal values for young subjects are used as criterion for the assessment abnormal findings have been reported in between 50 and 85 % of clinically healthy subjects above 70 years of age (Lepeschkin 1951, Fisch et al. 1957, Simonson 1961). The most common findings vary in different studies, comprising ectopic beats, A-V block I, low QRS voltage, Q wave changes, intraventricular conduction disturbances, depression of the ST segment and flattening or inversion of the T w

In the comprehensive investigation made by Nielsen (1963) the relationships between electrocardiographic findings at rest, clinical condition and autopsy findings were studied in 554 patients beyond 60 years of age. This study failed to demonstrate any electrocardiographic changes which could be definitely interpreted as related to old age per se and not to myocardial changes or coronary artery sclerosis observed at autopsy or to the clinical condition, e.g. incidence of arterial hypertension, heart failure or angina pectoris. It thus seems as if abnormalities in the resting electrocardiogram of elderly subjects would have clinical significance.

The dimensions of the cardiovascular system have been studied repeatedly in different age groups. Different results concerning the roentgenological size of the heart volume at different ages have been obtained, some studies showing no changes (Kjellberg et al. 1961b, König et al. 1961 and 1962) and others an increase in the volume with age (Maurea et al. 1955, Braun 1960). A constant blood volume with age has, however been observed in most studies based on reliable technique and comparable subjects in the different age groups (Cohn and Shock 1949, Sjöstrand 1949, Smith 1958, Yiengst and Shock 1962).

The mechanical events of the cardiac cycle were studied by Michel (1960) in 109 healthy subjects aged 8—86 years

by means of carotid artery sphygmograms. He observed a slight increase with age of the systolic time interval in relation to the total cardiac cycle, whereas in the study of Harrison et al. (1964) no significant changes with age were found. In the material of Michel there was a slight increase of the heart rate with advancing age, but Howell (1948) and Brandfonbrener et al. (1955) reported a slight decrease of the resting heart rate up to 80 years of age.

The effect of age on the cardiac out put at rest has been studied in normal subjects by many different metods. The marked decrease with age observed with the ballistocardiographic methods and the slight decrease with age observed with the indirect gas methods were discussed by Tanner (1949) and Brandfonbrener et al. (1955) By means of right heart catheterization and blood sampling from the right atrium or right ventricle using the Fick principle, a significant decrease of the cardiac output with age (up to 58 years) was observed by Cour nand et al. (1945) whereas Nickerson et al. (1947) found no significant age changes (up to 57 years) Using the dye dilution technique an extensive study was made by Brandfonbrener et al. (1955) on 67 male patients aged 19—86 years, who were free from signs of cardiovascular disease They observed a significant average decrease of cardiac output t rest by 1 % per annum and an average decrease of stroke volume by 0 7 per annum. I ccordance with these findings Rudewald (1962) observed decrease w th ge of the mean acceleration of the blood in the orta by differential pressure technique

The peripheral venous pressure was reported to be significantly lower in old age (Odenthal 1959) The increase with age of the peripheral vascular resistance was reported by Landowne et at. (1955) and the higher arterial systolic and diastolic blood pressures in old age have been confirmed in many studies (see e.g Master and Lasser 1961) However investigations of intracardiac pressures in healthy elderly subjects have so far not been published.

It should be evident from this brief survey that the effect of age on some of the parameters mentioned above has been studied repeatedly whereas a knowledge of intracardiac pressures and the effect of body position on cardiac output and stroke volume in old subjects is lacking. Moreover in previous studies only one or a few of the parameters ha e been measured simultaneously making it impossible to evaluate the relationships between the different data in old subjects. The present study was undertaken in order to obtain informa tion in these respects.

Present Investigation

The differences between old and young men are schematically summarized in tabl I

In th ecumbent position

Electroc rd'o r m The standard and precordial electrocardiogram was studied in 1?6 men aged 30—83 years (I) The incidence of quite normal findings de creased with rising age from 73 % (35 out of 45 cases) in the 30—39 to 31 %

Table I Schematic difference between old and young men concerning some circulatory data at rest generally in the supine position. "+" denotes higher mean values in old men, "0" denotes no difference and "—" denotes lower mean values. Re = recumbent S = sitting

Electrocardiogram	
Total score	+
Ectopic beats	+
QRS changes	+
ST T depressions	+
Heart volume all cases	+
Subjects with normal ECG	0
Total haemoglobin	0
Blood volume	0
Haemoglobin concentration	0
Heart rate	0
Mechanical systole	0
A V oxygen difference Re and Si	+
Cardiac output, Re	—
Stroke volume Re	—
Cardiac output and stroke volume Si	0
Pressures	
Brachial artery systolic	+
mean	+
diastolic	0
PCV mean	—
Pulmonary artery systolic	0
mean	0
diastolic	—
Right ventricular systolic	0
end-diastolic	0
early diastolic	0
Systemic resistance index	+
Pulmonary resistance index	+

(5 16) in the 70—83 age group The incidence of findings classified as abnormal or suspected abnormal increased simultaneously from 2 (1/48) to 31 % (5 16) The findings deviating from normal included ectopic beats incomplete and complete bundle branch blocks right and left axis deviations as well as ST junction depressions horizontal ST segment depressions and flattened T waves. The most common finding classified as abnormal or suspected abnormal was extreme left axis deviation (—30°—
—90 10 cases) which was most common in the higher age groups.

Only a few of the studied parameters of circulatory function were found to be related to the changes in the electrocardiogram at rest, classified as described under Methods. When heart volume was studied as dependent variable in *multiple regression* (III) it was observed that subjects with high scores (highest score = abnormal) for the total assessment of the electrocardiogram had larger heart volumes ($P < 0.05$) than the others, eliminating the influences of weight, blood volume and age, as had those with high scores for the ST segment ($P < 0.05$) or for ST segment and ectopic beats ($P < 0.01$) The subjects with high scores for the QRS complexes had slightly higher PCV and right ventricular end-diastolic pressures ($P < 0.05$) during supine exercise than the others (VIII) According to the regression coefficients the average filling pressures during exercise were 2 mm Hg higher than if only subjects with normal QRS complexes had been included in the study

Heart volume was determined in prone position in 74 men aged 50—83 years (III) and studied as dependent variable (y) in regression and multiple regression analyses. It was best correlated to weight and blood volume but also positively related to age (b = 2.69*) This last relationship however was lost

when the total assessment of the electrocardiogram at rest and during the exercise test (ECG_{total}) was included as independent variable in a multiple regression. The subjects with abnormalities in ECG_{total} thus had larger hearts ($P < 0.001$) than the others, and the relationship between age and heart volume was attributed to the increase with age of electrocardiographic findings deviating from the normal. It was similarly shown that successively larger heart volumes were observed the more marked the degree of ST depressions during the exercise test ($P < 0.001$) and the more frequent and abnormal the ventricular ectopic beats during the exercise test ($P < 0.05$). Heart volume was also related to electrocardiographic findings at rest (see above).

The mean heart volume in the subjects with normal ECG_{total} was 760 ml at a mean weight of 73 kg. The heart volume was on an average 150 ml or 19 % larger in the subjects with abnormal ECG_{total} than in those with normal electrocardiogram, with an increase of all heart diameters.

Eliminating the influences of weight, ECG_{total} and blood volume by multiple regression the residual standard deviation decreased to ± 12.0 % of the mean heart volume compared with the original standard deviation of ± 19.5 %.

The heart volume was also correlated to other circulatory data (VIII). During exercise the cardiac output increased more in relation to oxygen uptake ($P < 0.01$) in the old subjects with large heart volumes and these cases also had higher cardiac outputs in relation to oxygen uptake at rest and during exercise than the others ($P < 0.05$) as well as higher mean stroke volumes during exercise ($P < 0.05$). The subjects with large heart volumes also had a slightly higher PCV pressure at rest ($P < 0.05$) than the others.

Total haemoglobin and blood volume were determined in 74 men aged 30—83 years (III IV). None of these parameters, nor haemoglobin concentration, was primarily related to age or electrocardiographic findings to a probably significant extent. No influence of age could be found on the positive correlation coefficients between the dimensional parameters of the cardiovascular system, heart volume and blood volume, and between these and the functional parameter working intensity at heart rate 130 (W_{130}).

The average total haemoglobin was 785 g with a standard deviation of ± 15.0 of the mean. On multiple regression the residual standard deviation decreased to ± 7.9 % of the mean with the four positive independent variables, body weight, height, haemoglobin concentration and heart volume.

The average haemoglobin concentration was 13.3 g/100 ml with a standard deviation of ± 6.9 % of the mean. It was not primarily related to age or any of the measured data, except for the significant and positive relationships with total haemoglobin and weight.

The average blood volume was 5.90 l with a standard deviation of ± 13.9 %. At constant weight, height, haemoglobin concentration (negative regression coefficient) and heart volume, the residual standard deviation decreased to ± 8.0 % of the mean.

The blood volume was also significantly correlated to circulatory data obtained during heart catheterization (VIII) whereas corresponding correlations with total haemoglobin were less significant or not of probable significance. The old subjects with large blood volume thus had a higher cardiac output in relation to oxygen uptake at rest and during exercise than the others ($P < 0.01$) as well as a higher stroke volume during exercise ($P < 0.01$)

Heart rate was determined prior to the exercise test in 121 men aged 30—83 years (II) The average value was 67.0 ± 10.5 beats/min (± S.D.) There were no relationships of probable significance with age or electrocardiographic findings Subjects with low heart rates at rest also had low heart rates during submaximal exercise in comparison with the others, the correlation coefficient with W_{max} being significant or highly significant in all age groups (II) In the study of 26 subjects above 60 years of age (V) a low heart rate at rest was also associated with a low heart rate at maximal working intensity ($P < 0.05$) but not with a high W_{max} Slightly lower heart rates ($P < 0.05$) were also observed in tall subjects and in those with high values of total haemoglobin, blood volume and heart volume compared to the others (III IV)

Mechanical systole was measured in 20 men aged 61—83 years and 12 men aged 21 25 years (VI) The average value for the old men was 31.5 csec at heart rate of 65.5 beats/min and for the young men 33.4 csec at 66.8 beats/min The regression line for mechanical systole on heart rate had the same slope in old and young, and although the level of the line was slightly higher in the old men (+ 0.8 csec) this difference between old and young was not of probable significance ($P < 0.1$)

Cardiac output stroke volume and arterio-venous oxygen difference were determined by right heart catheterization in 17 men aged 61—83 years (VIII) The average values were 5.78 ± 1.15 l/min (± S.D.) 86.1 ± 12.2 ml and 44.8 ± 5.5 ml/l, respectively i.e. —24 %, —23 % and +18 % ($P < 0.001$) respectively in comparison with the average values observed under the same conditions in 23 young men with a mean age of 23 years (Holmgren et al. 1960 Bevegård et al. 1960) As the average heart rate was the same in old and young, the differences in stroke volume were equal to the differences in cardiac output. Only half of the decrease with age of cardiac output corresponded to the simultaneous decrease in oxygen uptake (—11 % $P < 0.001$) Therefore the cardiac output as related to oxygen uptake was significantly lower in old than in young persons, corresponding to the increase in arterio-venous oxygen difference

Within the group of old men the following relationships with these parameters were observed (VIII)

Cardiac output was higher in the cases with high heart rates and in those with high increases in oxygen uptake above predicted basal values, indicating the importance of "basal" conditions. According to the regression coefficient the "basal" cardiac output in these old men should be expected to be around 5.0 l/min Within this sample of old men

cardiac output could not be shown to be related to body size or age.

Stroke volume was more negatively correlated to arterio-venous oxygen dif ference than it was positively correlated to oxygen pulse. Neither stroke volume nor arterio-venous oxygen difference could be shown to be related to heart rate, oxygen uptake, increase of oxygen uptake above predicted basal value or to age.

Intracardiac and intravascular pres-su es were measured during heart cathe terization in 17 men aged 61—83 years (VII, VIII) and were compared with the values of previously studied young men (Holmgren et al. 1960 Bevegård et al. 1960) The average mean pressure in pulmonary arterial wedge position was slightly but significantly lower in the old men (—2.6 mm Hg) as was the mean value of the diastolic pressure in the pulmonary artery (—2.2 mm Hg) The average brachial artery systolic and mean pressures were signif icantly higher in the old men, + 25 mm Hg, respectively The other pressures were the same in old and young

The average sy temic and pulmonary resistance indices in the old men were 18 4 ± 4 3 mm Hg/l/min (± S.D) and 1 19 ± 0 57 mm Hg/l/min, respectively i.e. +30 % ($P < 0.001$) and +80 % higher ($P < 0.005$) than the values in young men

Within the group of old men it was observed (VIII) that the subjects w th the highest pulmonary capillary venous (PCV) pressures had slightly ($P <$ 0 05) higher systemic resistance indices, larger heart volumes, and lower cardiac outputs than the others.

Effect of body position

Heart rate was determined in standing position in 121 men aged 30—83 years (II) The average value was 82.1 ± 13.9 beats/min (± S.D) Like the findings in supine position the heart rate in standing position was not found to be related to age. Subjects with low heart rates in standing position were found to have larger heart volumes ($P < 0.01$) (III) and slightly more marked ($P <$ 0.05) electrocardiographic changes at rest and during the exercise test (ECG_{total} and ECG_{ST}) than the others.

The average increase in heart rate from supine to standing position was 15 1 ± 8.5 beats/min (II) The regression of this increase on age was significant, but the significance was due only to the subjects in the age groups 30—39 and 80—83 years as there was no relation to age between the ages 40 to 79 years.

As for the subjects with low heart rate in standing position the subjects with a low increase in heart rate from supine to standing position had slightly ($P < 0.05$) larger heart volumes and slightly more marked ($P < 0.05$) ECG_{total} and ECG_{ST} than the others.

The electrocardi gram was recorded in standing position in 126 men aged 30 —83 years (I) The incidence of moderate or marked orthostatic changes decreased w th age from 14 % (7/48) in the 30—39 to 2.5 % (2/78) in the 40—83 age group The subjects w th these changes had significantly higher heart rates in standing position (mean = 96 6 beats/min)

Cardiac output st oke volume and arterio-venous oxygen difference were

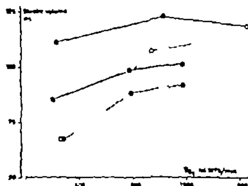


Fig. 1 Mean stroke volumes in relation to oxygen uptake ($\dot{V}_{O_2}$) at rest and during exercise in old (filled circles) and young men (open circles) in recumbent (full lines, n = 16 and 23 respectively) and sitting positions (broken lines, n = 6 and 9 respectively) (VII)

also determined in sitting position in 10 men aged 61—83 years (VII). The average cardiac output was significantly lower in sitting compared to supine position (—12 %) despite the significantly higher oxygen uptake (+38 %). The increase in arterio-venous oxygen difference on transition to sitting position was therefore marked (+59 %, P < 0.001). As the heart rate increased in sitting position (+14 %, P < 0.01) the decrease in stroke volume (—23 %, P < 0.001) was more pronounced than the decrease in cardiac output.

In the previously studied group of young men (Holmgren et al. 1960, Bevegård et al. 1960, 1962) the average increase in arterio-venous oxygen difference on transition to sitting position (+59 %) was the same as in the old men, whereas the increase in oxygen uptake (+20 %) was lower and the decrease in cardiac output (—26 %) more marked. The mean increase in heart rate (+20 %) was slightly higher and the decrease in stroke volume (—40 %) more marked than in the old men. On this account the only difference of probable significance between old and young in sitting position was the higher arterio-venous oxygen difference (+13 %) in the old men. The average oxygen uptake, cardiac output, stroke volume and heart rate were not found to be different (Fig. 1).

Intracardiac and intravascular pressures were also measured in sitting position in 10 men aged 61—83 years (VII). The right ventricular end-diastolic and early-diastolic pressures were significantly lower (4—5 mm Hg) in sitting than in supine position, whereas the differences of the right ventricular systolic and the pulmonary artery pressures were not even of probable significance. These lower pressures in sitting position at lower cardiac outputs and stroke volumes may be due either to real differences in effective pressures, to the fact that different reference points for zero pressure were used in the different positions, or to differences in the intrathoracic pressures.

In the old men significantly higher average diastolic (+9 mm Hg) and mean pressures (+8 mm Hg) were recorded in the brachial artery in sitting compared to supine position, whereas the higher systolic pressures (+8 mm Hg) were not of probable significance. The systemic resistance index in sitting position was on an average 21.3 ± 4.5 mm Hg/l/min (± S.D.), i.e. 22 % higher (P < 0.005) than in supine position.

These differences in pressures between supine and sitting position are mainly in accordance with the data of young men reported by Bevegård et al. (1960)

Discussion

Electrocardiogram The incidence of electrocardiographic changes deviating from the normal increased markedly with age. Although all the subjects in the present study were considered healthy from the clinical point of view the older subjects should be expected to have more marked coronary sclerosis than the younger (Lober 1953) and a higher incidence of myocardial sclerosis. The most frequent changes in the electrocardiogram concerned the QRS complexes and in 10 cases extreme left axis deviation was present. This condition has recently attracted interest and in 9 out of the 10 subjects in the present study the findings were consistent with peri-infarction block described by Grant (1956) In the study of Elliot et al. (1963) on apparently healthy men aged 23—61 years, extreme left axis deviation as single electrocardiographic finding was shown to be associated with present or later developing cardiovascular disease. In the present material the abnormalities of the QRS complexes, as classified in this study could only be shown to be slightly related to the filling pressure of the heart during exercise and not to any of the other studied variables.

Effect of age on cardiovascular dimensions With rising age the occurrence of atrophic processes (Bourne et al. 1961) will tend to reduce the heart weights, whereas contributory causes of cardiac hypertrophy such as arterial hypertension heart failure and coronary sclerosis have the opposite effect. In the present material the slight increase in heart volume with age was found to be associated with electrocardiographic changes rather than with age or with the level of the arterial blood pressure. In a previous paper (I) it was discussed why the ST depressions during the exercise tests were supposed to be signs of coronary insufficiency It was also suggested (III) that the ST depressions as signs of coronary insufficiency might be the cause of the increase of the heart volume. In the comparable small group of old men who were studied by heart catheterization, however there was no relationship of probable significance between heart volume and electrocardiographic changes. In this group the subjects with the largest hearts also had the largest stroke volumes during exercise and the most marked increase during exercise of cardiac output in relation to oxygen uptake. The lower stroke volume in relation to heart volume compared to young men may be associated either with slight increase of the muscular volume or a slight increase of the residual blood within the heart.

The volume of the aorta and larger arteries, the arterial compression chamber has been found to increase with age, around 300 ml between 20 and 65 years of age (Wetzler and Böger 1939) The total blood volume, however has not been found to be changed with age nor the extracellular water (Olbrich et al. 1957) This may partly be associated with the fact that the increase in aortic volume with age is too small to be de-

tected, or to the fact that the blood volume in the more peripheral parts of the body decreases simultaneously with the decrease of the lean body mass (Shock et al. 1953 Olbrich et al. 1957)

Effect of age on central circulation The central circulation in old compared with young subjects is characterized by a lower stroke volume which is not compensated by an increase in heart rate. This leads to a reduction of the cardiac output in old age, which is more pronounced than the decrease of the basal metabolic rate. Consequently the arteriovenous oxygen difference is higher in old than in young subjects and the circulation may be termed hypokinetic. The decrease in cardiac output is simultaneous to an increase of the systemic resistance which, however is more pronounced than the decrease in cardiac output. This leads to a higher arterial pressure in old subjects. In the pulmonary circulation the increase in resistance is equivalent to the decrease in cardiac output, giving an unchanged pressure drop over the pulmonary capillaries.

The decrease of the cardiac output with age does not seem to be proportional throughout the body as the reported values for the reduction of renal blood flow with rising age (Davies and Shock 1950) account for half or one third of the total decrease although the renal blood flow in young subjects only accounts for around one fifth of the total cardiac output. The renal blood flow thus decreases more with age than the total flow in the other parts of the body

The external left ventricular work per heart beat roughly calculated as stroke volume x brachial artery mean pressure reduced by mean PCV pressure, was on an average 11 % lower in the presently studied group of old compared to previously studied young men. The mean PCV pressure was, however also slightly lower in the old men so it cannot be definitely stated whether or not the old subjects had a lower mean stroke work in relation to filling pressure than the young.

Effect of body position at different ages The lesser orthostatic changes in central circulation in the old men was evidenced by the fact that in sitting position the mean cardiac output and stroke volume were not significantly different from the values in young men although marked differences were present in supine position. These differences in young and old men may at least partly be associated with the fact that the veins are more rigid in old age (Bourne et al. 1961) A certain increase in hydrostatic pressure should then cause a smaller volume increase of the veins in old age and a smaller decrease of the central blood volume in sitting position.

CHAPTER III

Adaptation of the Circulation during Exercise

Previous Investigations

In conjunction with physical exertion a higher frequency of ectopic beats and ST T depressions in the electrocardiogram has been recorded with rising age (Mazer and Reisinger 1944 Silver and Landowne 1953 Simonson 1953 Åstrand 1958 b Lepeschkin and Surawicz 1958, Rumball and Acheson 1960 Bellet et al. 1962) In only few of these studies, however has the recording been performed both during and after exercise and only few apparently healthy subjects above 60 years of age have been investigated.

In subjects of different ages the same mean values of heart rate or oxygen uptake at corresponding submaximal work loads on bicycle ergometers ha generally been reported (Åstrand 1958 a, 1960, König et al. 1961 1962, Grimby and Söderholm 1963 Hollman 1963) whereas the findings have been more variable with other types of exercise.

At maximal working intensity however both heart rate and oxygen uptake have been found to be successively lower with rising age in adults, at least above 30 years of age (Robinson 1938 Valentin et al 1955 Mitchell et al. 1958 Åstrand 1958 a, 1960 König et al. 1961 1962 Hollman 1963) The underlying physiological factors are not well understood.

A decrease with age of the arterial lactate concentration after maximal exercise has been reported (Robinson 1938 Åstrand 1958 a, 1960) whereas in most studies the mean lactate concentration during or after the same work loads has been higher in old subjects than in young (Åstrand 1958 a, 1960, Holmgren and Strandell 1959 Holmgren and Strom 1959) or of the same magnitude (Carlson and Pernow 1961) After the same exercise of short duration old subjects have shown higher lactate levels than young (Andersen 1959)

The brachial artery pressures measured indirectly during supine exercise were found to increase more t increasing work loads in old than in young men (König et al. 1962) Concerning intracardiac pressures, cardiac output and stroke volume during exercise no data relating to old men have been reported so far except the preliminary report by Granath et al. (1961)

The findings in different age groups as regards heart rate and oxygen uptake at submaximal and maximal working intensity are thus fairly well known at present, whereas the other functions are less known or have not been studied before. The present investigation was undertaken in order to study all these circulatory parameters in a group of healthy men up to the 9th decade to evaluate the effect of body position on some of the functions, and to study the interindividual relationships between the different circulatory data in order to obtain a more complete picture of the central circulation during exercise in old men.

Table II Schematic differences between old and young men concerning some circulatory data during sitting (Si) and recumbent (Re) exercise "+" denotes higher mean values in old men, "0" denotes no difference and "—" denotes lower mean values in old men at the same work load or heart rate.

Electrocardiogram, Si	
Total score	+
Ventricular ectopic beats	+
Supraventricular ectopic beats	+
ST depressions	+
Oxygen uptake Si and Re	0
Heart rate Si and Re	0
W_{130} and W_{170}, Si and Re	0
Arterial lactate conc., Si	+
Excess lactate conc., Si	+
Mechanical systole Re	+
A-V oxygen difference Si and Re	+
Cardiac output, Si and Re	—
Stroke volume Si and Re	—
1) Pressures, Re	
Brachial artery systolic	+
mean	+
diastolic	0
PCV mean	+
Pulmonary artery systolic	+
mean	+
diastolic	+
Right ventricular systolic	0
end-diastolic	+
early diastolic	+
1) Systemic resistance index	+
1) Pulmonary resistance index	+

1) At the best test load, which was on an average 29 lower in the group of old men.

Present Investigation

The differences between old and young men are schematically summarized in table II

The electrocardiogram was recorded during and after maximal or close to maximal exercise in sitting position in 126 subjects aged 30—83 years (1) The incidence of quite normal findings decreased with rising age from 54 % (26/48) in the 30—39 to 6 % (1/16) in the 70—83 age group. The incidence of findings classified as abnormal or suspected abnormal increased simultaneously from 6 % (3/48) to 75 % (12/16) The findings deviating from normal included both ST—T depressions and ventricular and supraventricular ectopic beats, the incidence in the 70—83 age group being 38 % (6/16) 31 % (5/16) and 31 % respectively The increase with age of abnormal findings is illustrated in fig 2.

Most of the ectopic beats were recorded during exercise, whereas the most marked ST depressions were generally recorded after exercise. Both the ventricular and supraventricular ectopic beats were highly significantly correlated primarily to the ST depressions when the classification system described under Methods was used ($r = 0.34$ and 0.44 respectively) This was, however due to the fact that the incidence of all types of abnormal findings increased with age. When the influence of age was eliminated there were no relationships of even probable significance ($r = 0.11$ and 0.06 respectively) A negative relationship of probable significance between body height and ST depressions was noted, the subjects with the most marked ST depressions being slightly shorter than the others. This relationship was not lost when age was included as independent variable which might suggest a slight relationship between body type and incidence of electrocardiographic changes consistent with coronary insufficiency

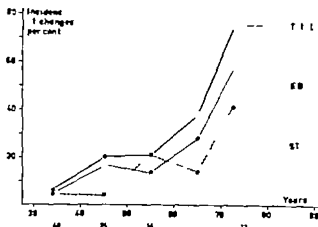


Fig. 2. Incidence of individuals with ECG findings classified as abnormal or suspected abnormal during exercise test in different age groups (I).
n = number of subjects,
ST = individuals with ST depressions,
EB = individuals with ectopic beats,
Total = individuals with ST depressions and/or ectopic beats.

The only significant relationships observed between electrocardiographic findings and other circulatory data were the one with heart volume (III) reported in chapter II and the lower increase in PCV pressure during exercise in the cases with the most abnormal ventricular ectopic beats.

Oxygen uptake was determined during submaximal exercise in sitting position in 24 men aged 60—83 years (II). By computing partial correlation coefficients it could be shown that at 600 kpm/min the subjects with the highest heart rates had the lowest mechanical efficiency ($P < 0.01$). The highest oxygen uptakes were recorded in the subjects with the highest heart rates ($P < 0.05$) and in those with the heaviest body weights ($P < 0.05$). No relation between oxygen uptake or mechanical efficiency and age or arterial lactate concentration could be shown.

Oxygen uptake was determined at maximal working intensity in sitting position in 19 subjects aged 61—83 years (V). It decreased significantly with age, on an average 0.38 l/min for 10 years increase in age.

Heart rate was determined during submaximal sitting exercise in 121 men aged 30—83 years (II). There were no significant relationships between age and heart rate at the different work loads, nor therefore between age and the working intensities at different heart rates (W_{130} and W_{170}). The coefficients of variation for a single determination of W_{130} and W_{170} were studied in the subjects above 60 years of age and found to be 9.5 % and 5.5 % respectively.

The old and young subjects showed the same linear increase of heart rate at increasing work loads. They did not differ concerning heart rate steady state at submaximal work loads. At the heaviest work load the mean 2—6 min heart rate increase was the same in old and young, but the heart rate at that load was successively lower with increasing age. During exercise there was an approximately linear rise of the mean 2—6 min heart rate increase with rising heart rate, although the individual

curves showed large variations concerning linearity. The correlation coefficients between W_{130} and W_{170} and body weight, height, heart rate at rest supine and standing, 2—6 min heart rate increase at the heaviest load and anamnestic degree of physical activity were not significantly different in the different age groups.

In 74 subjects aged 30—83 years the relationships between W_{130} and other circulatory data were more extensively studied (III, IV). W_{130} was highly significantly related both to blood volume, total haemoglobin and heart volume, but the lowest residual standard deviation was obtained by eliminating the negative influence of the heart rate at rest in standing position together with the positive influences of weight and anamnestic degree of physical activity. Neither age nor electrocardiographic findings were of probable significance.

Heart rate and work load at maximal working intensities will be discussed in Chapter IV.

Arterial lactate and pyruvate concentration were determined during sitting exercise in 6? men aged 30—83 years and 7 men aged 66—73 years, respectively. Under the conditions of the exercise test a very close correlation ($r = 0.99$) was observed between excess lactate reflecting the anaerobic metabolism in the working muscles (Huckabee 1958) and lactate concentration, excess lactate being equivalent to lactate reduced by mE/L. This relationship was similar to data previously given for young men (Carlson and Pernow 1961) and suggests that lactate concentration can be used for studying anaerobic metabolism during this exercise test.

The lactate concentration during exercise showed a significant and positive skewness with a mean greater than the median. When the log lactate values were used there was no skewness of probable significance. Because of the skewness, and because the logarithms of the lactate concentrations increased approximately linearly with rising heart rate or work load during the exercise test, the log lactate values were preferred in most calculations. By partial correlation studies it could be shown both in young and old men that log lactate concentration during exercise was significantly related to heart rate (relative work load) even after eliminating the influence of work load, whereas the relationship with work load (absolute work load) was lost when the influence of heart rate variations was taken into account. The close relationship between heart rate steady state and lactate concentration during exercise was evidenced by the significant correlation between log lactate and 2—6 min heart rate increase at the load, which was not lost after eliminating the influence of variations in heart rate.

The regression lines for log lactate concentration during exercise on heart rate in the different age groups were on a successively and significantly higher level with rising age although the individual variations were large. The higher lactate values during exercise in the old men compared with the young might have been connected with increased lactate production in the old men, decreased lactate elimination in the body or a smaller distributional volume for lactate as discussed previously (II).

Mechanical systole during supine exercise was studied in 20 men aged 61—83 years and 12 aged 20—25 years (VI). During exercise with stepwise increased work loads the duration of mechanical systole at first decreased approximately linearly with increasing heart rate, both in old and young men. When observations at heart rates above 150 beats/min were included, a significant curvilinearity was recorded in both age groups with successively lesser decrease of mechanical systole. At rapidly increasing heart rates during exercise or rapidly decreasing heart rates after exercise the changes in duration of systole were slower in both age groups than the changes in duration of diastole. The regression line of mechanical systol on heart rate at rest and during exercise up to heart rate 150 beats/min was at a slightly but significantly higher level (on an average 0 7 csec) in the old than in the young men, whereas the slopes of the lines were similar

Both for the young and the old men the verage mechanical diastole was shorter than the systole at maximal or near maximal working intensity As the mechanical systol during exercise was only slightly longer in the old men than in the young, the major fraction of the differences in maximal heart rate between young and old could not be associated with changes of the systolic or diastolic time intervals. The relationship between mechanical systole and maximal heart rate will be further discussed in chapter IV

Cardiac output st oke vol me and arterio-venous oxygen difference were determined in supine position by right

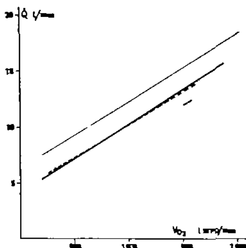


Fig 3 Regression lines for cardiac output (Q) on oxygen uptake ($\dot{V}_{O_2}$) in old (heavy lines) and young men (thin lines) in recumbent (full lines) and sitting positions (broken lines) (VII)

heart catheterization in 16 men aged 61—83 years (VII, VIII) In order to study the effect of age the data of the old men were compared with previously reported values of young men (Holmgren et al. 1960 Bevegård et al. 1960) During exercise the increase of cardiac output in relation to the increase in oxygen uptak was the same in old and young men (fig 3) As th old men had lower cardiac outputs and higher arterio-venous oxygen differences at rest than the young, similar differences were present during exercise. The cardiac output was on an verage 2.0 l/min lower in the old men t any level of oxygen uptake. The relationship between heart rate and oxygen uptak was the same in old and young men so that the lower cardiac output in the group of old men corresponded to lower stroke volumes (—14 % at the heaviest load, fig 1)

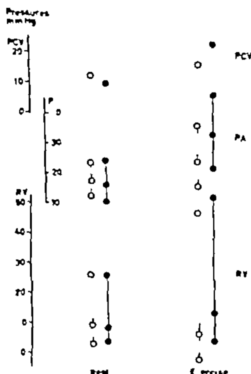


Fig 4 Mean values for pressures at rest and at the heaviest work load in recumbent position in 17 old (filled circles) and 23 young (open circles) men (VII) From top to bottom mean PVC pressure pulmonary artery systolic mean and diastolic pressures and right ventricular systolic, end-diastolic and early diastolic pressures.

Within the group of old men the following relationships with these parameters were observed (VIII)

During exercise the cardiac output increased more in relation to oxygen uptake in the cases with large heart volume at rest and high PCV pressure during exercise This suggests that no heart failure was present.

Subjects with small stroke volume during exercise small blood volume and heart volume low weight, low cardiac output and high arterio-venous oxygen difference at rest had a lower cardiac output in relation to oxygen uptake at rest and during exercise than the others. The subjects with the most pronounced hypokinetic circulation at rest were thus also more hypokinetic during exercise than the others.

Stroke volume during exercise was higher in the cases with high values of blood volume, body surface area, W_{130}, W_{max} heart volume, vital capacity and maximal voluntary ventilation (MVV) When blood volume and MVV were combined as independent variables, the residual standard deviation for stroke volume during exercise decreased to ± 5 2 %

The increase in stroke volume from the first to the second work load was positively influenced by the degree of physical activity according to case history and negatively by the increase in heart rate from the second to the sixth minute at the second load. The lowest residual standard deviation corresponded to an error for a single determination of stroke volume during exercise of ± 3 7 %

Intracardic and intravascular pressures were measured during right heart catheterization in supine position in 16 men aged 61—83 years (VII VIII) and compared with data from the previously mentioned group of young men (fig 4) The heaviest work load was lower in the old than in the young men (average oxygen uptake = 1 46 l/min compared to 2.06 l/min) as was the cardiac output (13 1 l/min compared to 18.5 l/min) and the heart rate (130 beats/min compared to 157 beats/min) Despite the lower work loads and cardiac outputs at the

heaviest load the systolic and mean pressures in the brachial artery were higher ($P < 0.001$) in the old men (+ 32 and + 19 mm Hg, respectively) as were the end-diastolic pressures in the right ventricle (+ 7 mm Hg) the systolic (+10 mm Hg) diastolic (+ 6 mm Hg) and mean pressure in the pulmonary artery (+ 9 mm Hg) (fig 4) The early diastolic pressures of the right ventricle were significantly higher in the group of old men (+ 5 mm Hg) as were the individual mean values for the PCV pressures at the two work loads (+ 7 mm Hg) The subjects with the highest right ventricular end-diastolic pressures during exercise also showed marked early diastolic dips.

The resistance indices of the pulmonary and systemic circulations were significantly higher in the old than in the young men, and more so than at rest. The increase in brachial artery systolic pressure during exercise in relation to cardiac output was significantly more marked in the group of old men (fig 5) Including the values at rest the brachial artery diastolic pressures were slightly but significantly higher in relation to cardiac output in the old than in the young men (fig 5)

The PCV pressures given above were recorded *after 2 minutes exercise at the* load. A significant decrease of the PCV pressure (on an average 4 mm Hg) from the second to the seventh minute of exercise at the different loads was observed in the 9 cases in which it was studied

Both at rest and during exercise there was good agreement between the "a" wave and the mean pressure in pulmo-

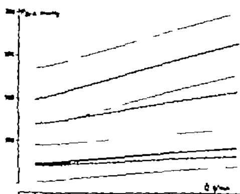


Fig 5. Systolic (S) and diastolic (D) pressures in the brachial artery in relation to cardiac output (Q) at rest and during exercise in recumbent position in 16 old men (VII). Regression lines (heavy lines) ± 95 confidence belts for single observations (thin lines). Broken lines denote regression lines in young men.

nary arterial wedge position. Variations of the mean PCV pressure were thus presumed to be a good measure of the variations in filling pressure of the left ventricle in these men, both at rest and during exercise.

Within the group of old men the following relationships with the pressures were observed (VIII)

The PCV pressure and the right ventricular end-diastolic (RV_{De}) pressure during exercise were positively related to degree of physical activity and to degree of abnormality of the QRS complexes in the ECG The increase of the PCV pressure during exercise was lower in the cases with frequent ventricular ectopic beats than in the others.

The pulmonary ventilatory function significantly and positively influenced the PCV and RV_{De} pressures during exercise The lowest pressures during

exercise were observed in the cases with the highest values for FRC/TLC and RV/TLC the lowest values for FEV and the most marked increases during exercise of the respiratory variations in the pulmonary artery systolic pressure i. e. in cases in which the highest airway resistances could be expected.

Individual changes in PCV and RV pressures during exercise were associated with corresponding changes of arterial pulse pressures during exercise and thus the ventricles of these old men seemed to function in accordance with Starling's law of the heart.

Effect of body position

Oxygen uptake was measured during exercise in both sitting and supine position during right heart catheterization in 8 subjects aged 61—83 years (VII) but no differences of probable significance were observed.

*Heart rate was measured during exer*cise in both sitting and supine position in 24 men aged 60—83 years (II). There were no significant systematic differences between the two postures, which is in agreement with the findings in young men (Bevegård et al. 1960). The variation of the differences between heart rate in sitting and supine position was the same as wh ring two tests n sitting posit

Arterial lacta on was de rmined during th sitting nd upine posi n aged 61 83 ears (II) to be significantly higher er cise has been ng

males (Holmgren 1959 Bevegård et al. 1963)

The electrocardiogram was recorded during and after maximal exercise in both sitting and supine position in 27 men aged 60—83 years (I). Although the maximal heart rate and work load was lower during supine exercise significantly more marked ST depressions and higher incidence of supraventricular ectopic beats were recorded under these circumstances. The higher incidence of supraventricular ectopic beats during exercise *in supine position may be attrib*uted to the higher filling pressures of the heart in this position. The increased degree of ST depressions in the supine position may presumably be connected with the higher stroke volumes compared to the values in sitting position, together with the probably reduced perfusion pressure over the coronary vessels (higher filling pressures for the right and left ventricles but unchanged brachial artery pressure in supine position).

Cardiac output stroke volume and arterio-venous oxygen difference were determined during exercise in both sitting upine position in 9 men aged 61— (VII). On transition st and at increasing ad position, the ave ase output in relatio en the same as th in ion (fig 3). A e it was lower r ous oxygen n tting present on a e 1 min el

In previously studied groups of young men (Bevegård et al. 1960 1962) the difference between cardiac output in sitting and supine position was more marked, on an average 2.0 l/min (fig 3) The difference between old and young men concerning cardiac output during exercise was thus less pronounced in sitting position.

The stroke volume in sitting position increased on an average by 32 % ($P <$ 0.005) on transition from rest to exercise (fig 1) With further increase of the work load the mean increase in stroke volume (+ 3 %) was not significant ($P >$ 0.05) Although this increase in stroke volume from rest to exercise was more marked than the 16 % increase in supine position, the stroke volume was significantly lower during exercise in sitting than in supine position, on an average 8 % at the heaviest load.

In the previously studied groups of young men the average stroke volume increased more on transition from rest to exercise in sitting position than in the group of old men (fig 1) and at the heaviest load it was 19 % higher in the young than in the old men.

Intracardiac and intravascular pressures were measured during exercise both in sitting and supine position in 9 men aged 61—83 years (VII) The brachial artery pressures were not significantly different in the two positions, but as the cardiac output was lower in sitting position, the systolic and mean pressures were significantly higher in sitting position in relation to cardiac output, the mean difference in pressure amounting to + 13 and + 9 mm Hg, respectively The systemic resistance index was thus significantly higher in sitting than in supine position, on an average 12 % at the heaviest load.

In sitting position all the average values of the pressures in the right ventricle and pulmonary artery were significantly lower (3—8 mm Hg) than in supine position. Different explanations of these lower pressures in sitting position were given in chapter II.

Similar differences in pressures between old and young men were observed during exercise in sitting position as were described above for exercise in supine position.

Discussion

The questions to be discussed are presented under the following headings.

1 *What information may be expected from exercise electrocardiograms in healthy elderly men?*

The findings relate particularly to two types of changes, ST depressions and ectopic beats. The reasons why the ST depressions might be regarded as signs of coronary insufficiency were discussed in detail in paper I and will not be repeated here. It will only be mentioned that the mortality in coronary artery diseases observed in follow-up studies was found to be closely related to ST depressions recorded after exercise (Robb et al. 1956 and others) In the present study the incidence of abnormal ST depressions increased with age and was much higher during exercise than the incidence at rest. The ST depressions could only be shown to be significantly related to the heart volume at rest and not to the central circulation data. Thus

the comparably small variations in the ST segment within the present material did not significantly affect the function of the heart as has been observed in patients with angina pectoris (Müller and Rørvik 1958). Due to the interindividual variations in maximal physical working capacity it may be stated that when the aim of an exercise electrocardiogram is to assess the presence or absence of signs of coronary insufficiency in an individual case with all the inherent sources of error the test should preferably be a maximal one.

The frequency of the other type of changes in old age, the ectopic beats, stresses the importance of a repeated control of the electrocardiographic changes during exercise also at submaximal work loads. If an elderly subject is forced to complete a predetermined work load without electrocardiographic control the chance of precipitating a dangerous arrythmia should be much higher than when performing a test as in the present study. The supraventricular ectopic beats were not found to be significantly related to any of the other circulatory data, and the ventricular ectopic beats only to the increase of PCV pressure during exercise. The positive relationship of probable significance between heart volume and the occurrence of ventricular ectopic beats during the exercise test might however be in accordance with the findings of Nielsen (1963) [illegible] observed an increased incidence of ventricular ectopic beats at rest in cases with clinical symptoms of coronary sclerosis and in cases in which fibrosis and infarction were observed at autopsy.

2. *Have interindividual variations in heart rate response during exercise the same significance in old and young men?*

In accordance with most previous studies the mean heart rates and oxygen uptakes at submaximal work loads were not affected by age in the present investigation and the random variation was the same in the different age groups. Nor were variations with age observed concerning the linearity of heart rate on work load at increasing loads. In both old and young the steady state of the heart rate during exercise was related to the degree of anaerobic metabolism measured as arterial lactate concentration.

This suggests that the load on a bicycle ergometer can be used as equivalent to oxygen uptake with the same accuracy in healthy old and young men. Due to the lower maximal heart rate during exercise in old age it was found to be more advantageous to determine the working intensity at a lower heart rate than 170 beats/min (e.g. at 150 or 130 beats/min), which is otherwise used in this laboratory (Sjöstrand 1960). Thereby long extrapolations and extrapolations above maximal heart rates were avoided and W_{130} could be determined at submaximal testing in the 8th and usually also the 9th decade. In the present study W_{130} was used to depict the heart rate response during exercise and

an estimate of the oxygen pulse during exercise this measure was found to be as closely related to heart volume, total haemoglobin and blood volume as W_{170}. The relationships between W_{130} and the dimensions of the cardiovascular system were not affected by age in subjects with

normal exercise electrocardiograms. Nor were differences between old and young observed regarding the correlations between W_{130} and other anthropometric data.

With regard to all the abovementioned factors the significance of the heart rate response during exercise seems to be equivalent in old and young men. However the relationship between the maximal physical working capacity and W_{130} or other measures of the heart rate response during submaximal exercise is not the same in old and young and the reasons why W_{130} could not be used as an estimate of the maximal working capacity in these old men will be discussed in chapter IV

3 *What are the effects of age on central circulation during exercise?*

Like the findings at rest the circulation during supine exercise in old men is also hypokinetic, with higher arterio-venous oxygen differences and lower stroke volumes and cardiac outputs than in young men. The higher systemic resistance and arterial blood pressure in old age is even more pronounced than at rest.

The fact that the increase in cardiac output in relation to oxygen uptake during exercise was the same in old and young men should suggest that the increase of blood flow to the working muscles was also the same unless the decrease of flow to other parts of the body was different in old and young. Similar (Carlson and Pernow 1961) or only slightly higher values (Strandell 1962) of the arterio-venous oxygen difference of femoral venous blood during exercise have also been found in old compared to young men.

The findings of higher intracardiac pressures in the old compared to the young men, illustrated in fig 4 may raise the question whether the pressure differences are real or are associated only for instance, with a higher mean intrathoracic pressure in the old men. This has not been investigated but seems very unlikely as, in the old subjects, findings denoting a high mean intrathoracic pressure were associated with lower than average filling pressures. Regarding the PCV pressures, however it cannot be definitely stated that they were higher in the old than in the young men at the time of the stroke volume determination. Both in old and young the PCV pressures were determined at the second minute at the load and it is not known whether or not they declined with time at constant load in the young men as well as in the old.

The higher pressures during exercise and lower stroke volumes in the old compared to the young men were discussed in detail in previous reports (VII VIII) With regard to the external stroke work at the heaviest load, the lower mean stroke volume in the old men was equivalent to the higher average mean pressure in the brachial artery so that the calculated average left ventricular stroke work was unaffected by age. Stroke work was roughly calculated from average values as stroke vol me x brachial artery mean pressure reduced by mean PCV pressure disregarding eventual differences between aortic and brachial artery pressures in old compared with young men. Although the average

filling pres ure of the left ventricle was higher in the old men at the same stroke work this does not necessarily mean that the myocardial function was decreased, as a pressure load may affect the heart differently than a volume load. Under experimental conditions isolated dog hearts consume more oxygen when work is increased by increasing pressure than by increasing output (Landowne and Katz 1950) In man the increased pressure load in cases of pulmonary stenosis increases the filling pressures of the right ventricle more than the increased volume load in cases of auricular septal defect at correspond ng levels of stroke work (Jonsson et al. 1957 Ikkos et al. 1962)

The h gher average right ventricular end-diastolic filling pressures during exercise in the old men were not associa ted with an increased pressure load. There is reason to believe however that the internal friction of the heart is increased in old age as a more rigid inter cellular collagenous connective tissue has then been observed (Kohn and Roller son 1959) This should unfa ourably affect the relationship between useful entricular work and total ventricular work and might contribute to the in creased filling pressures d ring exercise in the old men

In the present study the end-diastolic pres re i the right ventricle and the PCV pressure during exercise were not orrelated to heart volume or degree of ST dep es ion in the exercise electro rdiogram Nor were the lowest values of trok work observed in the cases with the largest he rts and the most marked electrocardiographic abnormalities, as in patient w th rterial h pertension (Var nauskas 1955) In patients with angina pectoris or previous infarction, high PCV pressures during exercise have also been found (Müller and Rorvik 1958) It has, however been observed that among subjects with angina pectoris and pre ious infarction those with the highest PCV pressures during exercise also have the lowest cardiac outputs during exer cise and generally show a decrease of stroke volume on transition to exercise (Malmborg 1963) In the present study on the contrary those with the highest PCV pressures had the most marked increase of cardiac output in relation to oxygen uptake during exercise

The influence of degree of physical activity and pulmonary ventilatory func tion on stroke volume and filling pressures during exercise will make it clear apart from all other reasons, that the findings in this group of old men may not be regarded as normal values for old men. In other groups of old men, d ffering from the present as regards physical activity and ventilatory function, different findings might be expected. This is similar to the findings in young men active athletes have higher filling pressures during exercise than ordinarily trained men (Bevegård et al 1963)

4 Wh t are the effects of body position at d ffer nt ages?

At a given working intensity the old men had the same mean oxygen uptake and heart rate in sitting compared to supine position, but lower cardiac out put, troke olume and intracardiac press res. The arterial lactat concentration, however wa significantly higher

during supine than during sitting exercise. The central circulation, as measured by the total cardiac output, was thus of no major importance for the degree of anaerobic metabolism in the working legs. The pertinent circulatory factor should be the distribution of cardiac output to the working muscles.

The findings in these old men are in accordance with previous findings in young men, except that the difference in cardiac output between supine and sitting position was less pronounced in the old men, as it was also at rest.

CHAPTER IV

Central Circulation as a Limiting Factor on the Maximal Physical Working Capacity

Previous Investigations

For work of 5—10 minutes duration it is generally considered that the maximal physical working capacity is mainly dependent on the aerobic capacity and can be measured as the maximal oxygen uptake during for instance bicycle work (Åstrand 1956). From 30 to 60 years of age the mean decrease in the total maximal oxygen uptake amounts in men to approximately 25—30 per cent (Robinson 1938, Valentin et al. 1955, Åstrand 1958 a, 1960, Konig et al. 1961, Hollmann 1963).

However it is less known in detail why the physical working capacity decreases with age and what factors limit it in old age. The decreased oxygen transporting capacity may either be due to the pulmonary, circulatory, muscular or metabolic function or to poor integration of these functions. The maximal pulmonary, circulatory and muscular functions are known to decrease with age. Concerning the circulatory function it is known that the average maximal heart rate during exercise in the sitting or standing position declines from 190—195 beats/min at 20 years of age to around 160—165 beats/min at 60 years of age (Robinson 1938, Åstrand 1958 a, 1960, Hollmann 1963). Also the stroke volume during sitting exercise is also lower in old than in young men, the maximal cardiac output is still more reduced in old age than the maximal heart rate.

A relationship between the physical working capacity and the dimensions of the cardiovascular system was observed by Kjellberg et al. (1949) in a material of normal subjects aged 8—55 years including children, women and men, some of whom were trained athletes. They found close correlations between W_{170}, used as a measure of the working capacity, heart volume and total haemoglobin. In a group of young girl swimmers the maximal oxygen uptake was found to be highly significantly related to heart volume, total haemoglobin, lung volumes and spirometric ventilatory data (Åstrand et al. 1963). The closest primary correlation was obtained with heart volume, and also in the subsequent multiple regression analyses this variable was the most significant one for predicting the maximal oxygen uptake. In a material of 60—5 year old males a correlation of probable significance was observed between the maximal oxygen uptake during steady state exercise in supine position and heart volume at rest.

The aim of the present investigation was to study in a group of old men the heart rate (HR_{max}) and work load at maximal working intensity (W_{max}) in different body positions. Furthermore the relationships were studied between HR_{max}, W_{max} and some parameters of circulatory, ventilatory and metabolic

function In order to evaluate which of these factors could be supposed to limit the physical working capacity

Present Investigation

HR_{max} and W_{max} in sitting position were determined in 27 men aged 61—83 years (V) The average values at the first test were 153 ± 17 beats/min (± S. D) and 818 ± 166 kpm/min, respectively and did not increase significantly when the tests were repeated HR_{max} decreased on an average by 15 beats/min for 10 years' increase in age, whereas W_{max} simultaneously decreased by 140 kpm/min. W_{max}, used as a measure of the maximal physical working capacity was highly significantly related to the maximal oxygen uptake in the 19 subjects studied.

HR_{max} and W_{max} in supine position were significantly lower than corresponding values in sitting position, the mean differences being 14 beats/min and 145 kpm/min, respectively In supine position the incidence of fatigue in the legs on interruption of the test was higher ($P <$ 0 02) than in sitting position.

HR_{max} and W_{max} during combined arm and leg work When the leg work in sitting position was combined with simultaneous arm work (cranking on another ergometer) HR_{max} and W_{max} increased significantly on an average 7 beats/min and 60 kpm/min, respectively

HR_{max} as dependent variable When HR_{max} in sitting position was studied as dependent variable (y) in regression analyses, the only significant relationships were the negative ones with log arterial lactate concentration at heart rate 130 (Log lact $_{130}$) age and W_{130}. A low HR_{max} was thus associated with high values of these parameters. When multiple regression analyses were performed with three independent variables, the lowest residual standard deviation was obtained with these three parameters together (± 6 1 % of the mean value compared to the original standard deviation of ± 10.0 %) By including a parameter obtained at maximal working intensity Log $lact_{max}$ slightly lower residual standard deviations were obtained, the lowest one being with the following five independent variables W_{130}*** Log $lact_{max}$** age** physical activity* and St st_{130}* (2—6 min heart rate increase at heart rate 130) A low HR_{max} was associated with a low value for Log $lact_{max}$ and a low score for the degree of physical activity (low score = lowest activity) All the other relationships were negative.

Body weight, height, heart volume, total haemoglobin, haemoglobin concentration, and blood volume were not of probable significance when tested alone or in combination with any of the other independent variables. Nor were any of the assessments of the electrocardiographic findings of probable significance. As parameters of lung volumes and ventilatory function the total lung capacity vital capacity functional residual capacity residual volume FRC/TLC RV/TLC, forced vital capacity FEV $_{1.0}$ FEV % maximal voluntary ventilation and respiratory rate at 600 kpm/min were tested as independent variables However none of them was of probable significance when tested alone or in combination with other variables.

In 70 men aged 61—83 years the relationship between HR_{max} and the mechanical systole was studied (VI). By partial correlation studies a slight relationship ($P < 0.05$) was observed between HR_{max} and the slope of mechanical systole on heart rate, the cases with the lowest HR_{max} showing the most marked decrease in the duration of mechanical systole at increasing heart rates.

Within the group of 17 old men studied by right heart catheterization (VIII) none of the variables used were found to be related to HR_{max}.

W_{max} as dependent variable. When W_{max} was studied as dependent variable (y) in regression analyses, the only highly significant relationship was the negative one with low arterial lactate concentration at 600 kpm/min (Log $lact_{600}$). The original standard deviation of ± 20 [illegible] of the mean then decreased to ± 13 [illegible]. Significant negative relationships were obtained with St st_{600}, St st [illegible] and age as single independent variables. A low W_{max} was thus associated with high values of these parameters. A slight positive correlation was found with W_{30} ($P < 0.05$).

When multiple regression analyses were performed with two or three independent variables, the lowest residual standard deviation was obtained with Log $lact_{600}$ and age [illegible] including a parameter for [illegible] maximal working intensity. Log lac[illegible] the residual variance decreased further with the five independent variables W_{30} [illegible] physical [illegible] St [illegible] Log $lact_{max}$ [illegible] A low W [illegible] associated [illegible] high [illegible] and

high age, all the other relationships being positive.

There was no correlation between W_{max} and the amplitude of the arterial calf pulsations when the influence of age was eliminated, but a probably significant primary correlation coefficient. Log $lact_{600}$ was not related to the arterial calf pulsations.

Within the group of 17 old men studied by right heart catheterization (VIII) none of the data concerning pressure and flow were related to W_{max} except for the probably significant relationship with the stroke volume at the second load. This relationship was lost, however, when the influence of age or Log $lact_{600}$ was eliminated. The negative relationship between Log $lact_{600}$ and stroke volume at the second load was of probable significance.

As for HR_{max}, the body weight, height, heart volume, total haemoglobin, haemoglobin concentration, blood volume, assessments of the electrocardiographic findings and the studied parameters of lung volumes and ventilatory function were not even of probable significance when tested alone or in combination with any of the significant independent variables.

Discussion

Contrary to the findings in young subjects no relationships were observed in these old men between W_{max} and the *dimensions of the cardiovascular system*, the heart volume and the blood volume. These findings suggest that other factors were more important for the maximal working intensity. Only slight po-

ative primary correlations were found with central circulation data such as stroke volume during exercise and W_{max}. There was no relationship suggesting that subjects with hypokinetic circulation during exercise (high total arteriovenous oxygen difference) had lower W_{max} than the others.

The closest relationship observed was the negative one between W_{max} and log arterial lactate at 600 kpm/min. According to the data in chapter II the lactate values should be expected to reflect the anaerobic metabolism in the working muscles, and the data suggest that this should be the main limiting factor on the maximal working capacity in the present material. The lack of relationship between the arterial calf pulsations and Log $lact_{600}$ should suggest that the anaerobic metabolism during exercise was not related to obstructive changes in the main arteries of the legs. The maximal working capacity representing the maximal aerobic capacity thus varied with the ability of the subjects to avoid accumulation of metabolites from anaerobic metabolism, and this ability could be assessed at submaximal work loads. The maximal physical working capacity measured as W_{max}, was accordingly related to peripheral factors, as has been suggested earlier regarding young subjects (Åstrand 1952, Cobb and Johnson 1963) and may have been associated with the muscular mass engaged in the exercise, the distribution of muscle blood flow the diffusion of oxygen from the capillaries into the muscle cells, and with metabolic cellular factors. Whether or not the same is true of young subjects cannot be stated, as no study has yet been made with the same technique. However as physically active subjects have been found to have similar cardiac outputs during exercise as sedentary or less physically active subjects, but lower values of arterial lactate concentration (Bevegård et al. 1963 Cobb and Johnson 1963) it is rather probable, although the relationships with the dimensions of the cardiovascular system are also high, suggesting a relationship with the central circulation.

The lower W_{max} and HR_{max} observed in supine compared to sitting position may probably be attributed to the possibility that smaller or less well-trained muscle groups were then engaged, besides the effect of hydrostatic counter pressure as previously discussed (V). This should also be in agreement with the observed higher values for arterial lactate concentration during supine exercise (II).

The higher values of W_{max} and HR_{max} recorded during combined arm and leg work compared to leg work alone showed that the values for sitting leg work did not represent the physiological maximal values, but only the maximal values under the conditions studied. The main reason for the higher values during combined arm and leg work seemed to be the effect of counterirritation, i.e. the sensations from the arms or the mental concentration on cranking with the arms seemed to suppress the sensations from the legs as discussed previously (V).

The present observation that the assessment of the electrocardiogram was without significance for W_{max} and HR_{max} is in agreement with earlier findings (Åstrand 1958 b). The lack of cor

relation between W_{max} or HR_{max} and lung volumes or measurements of pulmonary ventilatory function should indicate that ventilatory factors did not significantly limit the capacity for this type of physical exercise in the present material of old males. Of course this does not exclude the possibility of pulmonary limitation in some of the subjects. Besides, a slight effect of pulmonary limitation might already have been taken into account in the other significant independent variables. In the present study, however, there was no correlation of probable significance between the ventilatory parameters and any of the significant or probably significant independent variables. Nor were signs of pulmonary insufficiency present during maximal working intensity when arterial oxygen and carbon dioxide tension were studied in some of these men (Strandell 1959).

Exercise test for old men

Judging from the findings in this material the following suggestions for exercise tests on old men may be given.

When the primary interest is the study of the cardiovascular function of the subject in relation to body size and to the dimensions of the heart and the vascular system, the function may be estimated from the heart rates at submaximal work loads and measured, for instance, as W_{130}, since this parameter is related to the dimensional variables and mainly in the same way as in younger men. Because of the decrease of HR_{max} with age, W_{130} was determined in this study instead of W_{170}, which is generally used as functional parameter in this laboratory.

When, on the other hand, the primary interest is the study of the maximal capacity of the subject for exercise of moderately short duration, as in the present investigation, the prediction of this capacity from the heart rate during submaximal work loads seems to be of little practical value. Either the arterial lactate concentration should be studied during submaximal exercise or, better, a maximal test should be performed, for instance the simple one described here with determination of W_{max}.

General Summary and Conclusions

The circulation at rest and during exercise on a bicycle ergometer in both sitting and supine position has been studied in 140 healthy men aged 20—83 years, and a comparison has been made with previously known data for young men.

At rest

The old men had larger heart volumes in supine position than the young. This was related to the increased incidence with age of electrocardiographic findings deviating from the normal, both at rest and during the exercise test, and especially related to ST depressions and ventricular ectopic beats.

Blood volume, total haemoglobin and haemoglobin concentration were the same in old and young men. This was also true of heart rate and mechanical systole.

The central circulation in the old men showed, in supine position, a lower stroke volume than in the young subjects. The cardiac output was correspondingly decreased, as the heart rate was the same in old and young. Since the reduction of cardiac output was more pronounced than the decrease in oxygen uptake with age the arterio-venous oxygen difference was higher in the old than in the young men, i.e. the circulation was hypokinetic.

The increase of the systemic resistance with age was more pronounced than the reduction of cardiac output, leading to higher brachial artery systolic and mean pressures in the old men. In the pulmonary circulation the increase in resistance was equivalent to the decrease in cardiac output, corresponding to an unchanged pressure drop over the peripheral pulmonary vessels.

Pressures at right side heart catheterization were mainly the same in old and young men, but slightly lower values were observed for the mean pressure in pulmonary arterial wedge position and for the pulmonary artery diastolic pressure.

The orthostatic changes in central circulation were less pronounced in the old than in the young men, which eliminated the differences in stroke volume and cardiac output observed in supine position. In sitting position the average stroke volume and cardiac output were thus not different in old and young, but the arterio-venous oxygen difference was still slightly higher in the old men.

The increased arterial pressures, the decreased stroke volumes and the less pronounced orthostatic changes in the old men are discussed. It is suggested that these changes might be related to increased rigidity of the arterial and venous vascular walls in old age.

During exercise

The incidence of abnormal electrocardiographic findings during and after the maximal exercise test increased with age and was much higher than the incidence at rest. The findings consisted of ventricular and supraventricular ectopic beats, most often recorded during exercise and ST depressions. The reasons why the ST

relation between W_{max} or HR_{max} and lung volumes or measurements of pulmonary ventilatory function should indicate that ventilatory factors did not significantly limit the capacity for this type of physical exercise in the present material of old males. Of course this does not exclude the possibility of pulmonary limitation in some of the subjects. Besides, a slight effect of pulmonary limitation might already have been taken into account in the other significant independent variables. In the present study however there was no correlation of probable significance between the ventilatory parameters and any of the significant or probably significant independent variables. Nor were signs of pulmonary insufficiency present during maximal working intensity when arterial oxygen and carbon dioxide tensions were studied in some of these men (Strandell 1959)

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Phosphate disappearance from plasma and the renal handling of phosphate after intravenous loading in man

by

BENGT ARNER

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SUPPLEMENTUM 415

FROM THE MEDICAL CLINIC A, THE DEPARTMENT OF CLINICAL CHEMISTRY AND
THE CLINICAL PHYSIOLOGICAL LABORATORY LASARETTET LUND, SWEDEN

Phosphate disappearance from plasma and the renal handling of phosphate after intravenous loading in man

BY

BENGT ARNER

LUND 1964

Translated by
Mrs Kerstin Emmelin, B M

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BERLINGSKA BOKTRYCKERIET
LUND 1964

To my Wife and Family

CONTENTS

Abbreviations and symbols

GFR	glomerular filtration rate.
GFR_{In}	glomerular filtration rate determined by inulin clearance
GFR_{P}	glomerular filtration rate calculated exclusi ly from phosphate data in clearance experiments with falling phosphat concentrations.
Tm	maximal tubular reabsorption.
TmP	maximal tubular reabsorption of phosphate.
TmP_{I}	maximal tubular reabsorption of phosphat calculated as the difference between filtered load and excreted amount.
TmP_{II}	maximal tubular reabsorption of phosphat calculated from phosphat data.
V_{P}	apparent volume of distribution for phosphate.
t	time after end of phosphat infusion.
t_{mp}	midpoint of urine collection period, corrected for assumed renal tract delay
$t_1—t_2$	times t beginning and end of a urine collection period, corrected for ssumed renal tract delay
mg %	mg per 100 ml plasma.
y	plasma phosphat conce tration t time t.
y	plasma phosphat concentration t t_{mp}.
$y—y$	plasma phosphat concentrations t beginning and end of a urine collection period.
z_t	plasma inulin concentration t time t
$\bar{z}$	plasma inulin concentration t t_{mp}.
P_U	mean excretion of phosphate during urine collection period.
$\overline{In}_U$	mean excretion of inulin during a urine collection period.
BSA	body surface area.
M	mean alue
S.D	standard deviation.
S.E.M.	standard error of the mean.
n	number of observ tions.

Statistical methods

In the statistical evaluation of the data ordinary methods were used. (DAHLBERG, 1948, BONNIER & TEDIN 1957)

The significance of the difference between two means was evaluated us ing the t test. A difference was con sidered almost significant(*) signifi cant( ) and highly significant( ) when the corresponding levels of probability (P) were 0 05—0 01 0 01—0 001 and less than 0 001 respectively

When judging whether a regression coefficient differed from zero the same method and the same symbols were used.

INTRODUCTION

Our knowledge of the renal handling of phosphate is still incomplete. It is generally supposed that phosphate is reabsorbed from the primary urine in the tubules (see e.g. PITTS, 1963). Whether inorganic phosphate in plasma may be considered entirely ultrafiltrable or not has been extensively discussed. Experiments with artificial membranes (cellophane and collodium) suggest that plasma phosphate is completely ultrafiltrable (WALKER, 1933, BENJAMIN & HESS, 1933, HARRISON & HARRISON 1941, SMITH *et al.* 1943, ELLIOT *et al.* 1949 and others). In ultracentrifugation experiments phosphate was found to form complexes with plasma proteins and calcium. From such experiments some authors have concluded that only about 85 p.c. of the phosphate is ultrafiltrable (WALZER, 1960, LOKEN *et al.*, 1960).

LEVINSKY & DAVIDSON (195) and NICHOLSON & SHEPHERD (1959) maintained that phosphate is excreted by tubular secretion. This view was based on animal experiments. In man it has never been shown that excretion of phosphate can exceed the filtered load. The tubular reabsorption of phosphate can only be measured as the difference between filtered load and excreted amount and it is therefore impossible to decide whether the figure obtained is the result of tubular reabsorption only or of a combination of secretion and reabsorption.

If plasma phosphate concentration rises—for instance during infusion of a phosphate buffer solution—the reabsorption will increase and reach a maximum (see e.g. PITTS, 1963). The maximal tubular capacity for reabsorption of phosphate (TmP) has generally been regarded as characteristic for each individual—in the same way as the tubular maximum for glucose reabsorption—and should constitute a measure of a tubular function. This would imply that TmP does not vary with the filtration rate in one and the same subject. According to later investigations, however, TmP does vary with the filtration rate (LONGSON *et al.*, 1956, ANDERSON & PARSONS, 1963). In two investigations, one on dogs (SMITH *et al.* 1943) and one on human subjects (REYNOLDS *et al.*, 1960) it was impossible to find any Tm for phosphate at all. The methods used in these investigations are, however, open to

criticism (see e.g. PITTS & ALEXANDER 1944).

Simultaneous determinations of glomerular filtration rate and of the concentrations of phosphate in plasma and urine are required for a calculation of TmP. Furthermore, the reabsorption must be determined at several different, if possible constant, plasma concentration levels. To achieve this, a series of priming injections and sustaining infusions of phosphate have been given. It has, however, proved difficult to obtain such constant levels and in most experiments the concentration has been either rising or falling. Only few authors have succeeded in obtaining constant levels, for instance COHEN *et al.* (1956) and ANDERSON & PARSONS (1963). In a few cases TmP has been determined at falling concentrations after single injections (SMITH *et al.*, 1943, LAMBERT *et al.* 1947).

When studying TmP in large clinical series it would be very useful if phosphate could be given as a single injection or brief infusion of phosphate buffer solution and if both GFR and TmP could be calculated exclusively from phosphate data.

Calculating GFR and TmP from phosphate data has been tried by ANDERSON (1955) and LONGSON *et al.* (1956) but with conflicting results probably due to differences in technique. In both studies the calculations were based on GOVAERTS (1948) theory that the excretion is a linear function of the plasma concentration at levels where the reabsorption is constant. From the regression of the excretion on the plasma concentration GFR and TmP can thus be calculated. One condition for the application of this method is, however, that GFR as well as TmP remain constant throughout the experiment, as pointed out by SMITH (1951). Furthermore, only *one* mean value of GFR and *one* of TmP are obtained from all the data of the whole experiment. ANDERSON infused a phosphate buffer solution using a special technique in order to obtain linearly rising plasma concentrations. In his experiments, lasting 5—6 hours, the diuresis was very high (10—15 ml/min) and urine was collected during periods of 30 minutes by voluntary voiding. In a series of 5 normal subjects he found a good agreement between the values of GFR, calculated from phosphate data, and the mean value of inulin clearance, which was fairly constant.

The experiments of LONGSON *et al.* were carried out during phosphate infusion and the plasma concentration was falling slowly. In half of their experiments, where the glomerular filtration rate remained constant, they found a correspondance between GFR calculated from phosphate data and inulin clearance. In the other half where the inulin clearance varied greatly during the experiments, GFR could not be calculated from phosphate data. As already pointed out, TmP was found to vary with GFR.

It is reasonable to assume that under certain conditions the study of phosphate disappearance from plasma would allow the calculation of some parameter(s) determining the phosphate excretion through the kidneys.

Initial experiments led to studies of various methods for data analysis of disappearance processes, since the methods generally used in experimental medicine were found to be less suitable for the analysis of phosphate disappearance from the plasma.

The aim of this work was

1. to find a method suitable for data analysis of the disappearance of phosphate from the plasma after intravenous loading,
2. to study the phosphate disappearance in healthy subjects and patients in which the renal function was impaired to a varying degree, and
3. to study the relation between the renal handling of phosphate and the phosphate disappearance in clearance experiments on such patients.

CHAPTER I

A THEORETICAL DISCUSSION OF THE RELATION BETWEEN PHOSPHATE DISAPPEARANCE FROM PLASMA AND THE RENAL HANDLING OF PHOSPHATE AFTER INTRAVENOUS LOADING

Inorganic phosphate added to the blood by single intravenous injection or brief infusion is distributed to the plasma compartment and to an unknown number of other compartments. During the infusion, and for some time afterwards, the fall in concentration in the plasma is thus caused partly by diffusion to the tissues and partly by elimination through the kidneys. It seems reasonable to assume that after some time there will be a momentary concentration balance between the plasma compartment and other parts of the phosphate space. This is considered to happen with other substances, such as inulin, added to the blood by single injection (SHACHTER, FREINKEL & SCHWARTZ, 1950) After this momentary equilibrium a concentration gradient towards the plasma compartment will arise. When sufficiently long time has passed the distribution and elimination processes should be balanced so that the disappearance of phosphate from plasma will depend only on the function of the kidneys. The disappearance will then be determined by the glomerular filtration and the tubular reabsorption of phosphate The latter will be constant (TmP) If the concentration is still high enough. This means that there is a system of linearer Zufluss und exponentiale Elimination (DOST 1953) An equation for the relation between concentration and time in such systems has been deduced by TEORELL (1937)

A simple kinetic model for the distribution and elimination of phosphate can be set up It is similar to models constructed for such substances which, like for instance bilirubin (HÖRLEIN 1951) are constantly added to the plasma by endogenous production and simultaneously removed by a filtration, proportional to the plasma concentration.

The phosphate is assumed to be dispersed in a space, apparent volume of distribution (V_P ml) the size of which is assumed to be constant. The concentration in V_P is equal to the plasma concentration (y mg %) and the phosphate is assumed to be completely filtrable. The glomerular filtration rate (GFR ml/min) and the tubular reabsorption (TmP mg/min) are constant

In this kinetic model the velocity constant of disappearance (k) is deter

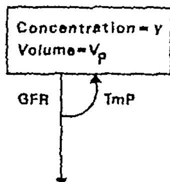


FIGURE 1 A kinetic model for the distribution and elimination of phosphat during late phase of the disappearance process after intra enous loading

mined by the filtered load per minute per unit load

$$k = \frac{GFR}{V_p} \cdot \frac{y}{y} = \frac{GFR}{V_p} \, min^{-1} \qquad (1)$$

The plasma concentration will fall towards a definite value (the asymptote constant, a mg %) where the filtered load equals the reabsorbed amount of phosphate

$$a = \frac{TmP \; 100}{GFR} \, mg \,\% \qquad (2)$$

An equation giving the plasma concentration as a function of time can be deduced from the kinetic model. Assume that the plasma concentration decreases with Δy mg % during a short time Δt. Since

loss in V_p = filtered amount − reabsorbed amount (3)

we get

$$\frac{\Delta y \; V_p}{100} = -\frac{GFR \; y \; \Delta t}{100} + TmP \; \Delta t \qquad (3\,a)$$

which gives

$$dy/dt = -\frac{GFR}{V_p}\left(y - \frac{TmP \; 100}{GFR}\right) \qquad (3\,b)$$

which by virtue of Eqs. 1 and 2 can be written as

$$dy/dt = -k(y-a) \qquad (3\,c)$$

From Eq 3 c it follows that dy/dt, e.g. the rate of disappearance (mg % /min) is a linear function of the plasma concentration (y) and that the inclination of the corresponding straight line measures the rate constant of disappearance (k min)

Integration of Eq 3 c gives

$$ln(y-a) = -k\,t + C, \qquad (4)$$

where C is the integration constant.

If $t = t$ and $y = y$ are inserted in Eq 4 we get

$$ln(y-a) = -k\,t + C. \qquad (4\,a)$$

Eqs 4 and 4 a give

$$ln\frac{(y-a)}{(y_1-a)} = -k(t-t\,) \qquad (5)$$

or

$$y = (y-a)\; e^{-k(t-t_1)} + a \qquad (5\,a)$$

If $t = 0$ and $y = y$ we get

$$y = (y-a)\; e^{-k} + a \qquad (5\,b)$$

where $(y-a)$ is a constant representing the y intercept of the curve $(y-a) = f(t)$ under the assumption that the conditions accepted for the model were valid during the whole process from zero time. If $(y-a) = b$ the final equation will be

$$y = b\; e^{-k} + a. \qquad (5\,c)$$

Thus we find that the disappearance of phosphate from plasma in the theoretical model is described by a simple equation with three constants The equation contains one exponential and one constant term Eq 5 shows that $\log(y-a)$ is a linear function of time and Eq 3 c that the derivative (dy/dt) is a linear function of the plasma concentration (y)

For substances which are filtered and reabsorbed we have

Filtered amount − reabsorbed amount = excreted amount (6)

In clearance experiments, where the excretion of phosphate is determined *during a number of urine collection* periods, the renal handling of phosphate can therefore be described by the equation

$$\frac{GFR\ \bar{y}}{100} - TmP = \bar{P}_U \qquad (6\ a)$$

where $\bar{y}$ denotes the average plasma concentration during one urine collection period with a mean excretion of phosphate $\bar{P}_U$ (mg/min)

Eq 6 a can be written as

$$\frac{GFR}{100}\left(y - \frac{TmP\ 100}{GFR}\right) = \bar{P}_U \qquad (6\ b)$$

or

$$GFR = \frac{\bar{P}_U\ 100}{(y-a)} \qquad (6\ c)$$

It should thus be possible to calculate GFR, TmP and V_P for each separate urine collection period from clearance determinations made during the fall in plasma concentration after a single injection, if the constants a and k could be determined by data analysis of the phosphate disappearance curve.

TmP is calculated from Eq 2

$$TmP = \frac{GFR\ a}{100} \qquad (2\ a)$$

and V_P from Eq 1

$$V_P = \frac{GFR}{k} \qquad (1\ a)$$

The accuracy of the kinetic model and the equations for calculating GFR, TmP and V_P may be tested in loading experiments of suitable design. The amount of phosphate administered would have to be large enough to give a maximal tubular reabsorption and the observation period sufficiently long to ensure that an equilibrium between distribution and elimination will be established.

In such loading experiments performed on normal subjects and patients with varying degree of renal damage, it would be possible to find out whether any part of the disappearance process can be described by empirical equations of the type here deduced (Eq 5 c) In clearance experiments, in which the excretion of phosphate is determined in a number of consecutive urine collection periods, GFR, TmP and V_P can be calculated entirely from phosphate data with the aid of Eqs 6 c, 2 a and 1 a It would thus be possible to find out to what extent these physiological entities remain constant during each individual loading experiment If inulin clearance determinations for each urine collection period were made simultaneously using constant infusion, a direct compar

ison could be obtained between GFR, calculated from phosphate data (GFR_P) and GFR as determined by inulin clearance (GFR_{In})

When GFR_{In} is known, the tubular reabsorption of phosphate can be calculated in the usual way as the difference between filtered load and excreted amount. It can then be tested whether the reabsorbed amount per 100 ml glomerular filtrate is constant throughout the disappearance process and corresponds to the asymptote constant (a) obtained by data analysis of the plasma phosphate concentration curve. Also for the velocity constant k a similar comparison can be made, as V_P for each urine collection period can be calculated without using the equation for the phosphate disappearance.

If y_1 and y_2 are the plasma concentrations in the beginning and the end of one urine collection period ($t_2 - t_1$) where the mean excretion of phosphate is P_U mg/min we get

$$\frac{V_P (y_1 - y_2)}{100 (t_2 - t_1)} = P_U \qquad (9)$$

or

$$V_P = \frac{\bar{P}_U (t_2 - t_1) \, 100}{(y_1 - y_2)} \qquad (9\,a)$$

The quotient of GFR_{In} over V_P calculated according to Eq 9 a, gives a value of the disappearance rate constant (k) which can be compared with the corresponding value obtained by data analysis of the phosphate disappearance curve.

Comparing different values for the constants k and a in this way has the advantage of being independent of any error made when collecting the urine.

Summary

On the basis of previous knowledge of the renal handling of phosphate a kinetic model is suggested for the distribution and elimination of phosphate during a late phase of a disappearance process when the tubular reabsorption is still at its maximum.

Assuming that inorganic phosphate in plasma is completely filtrable a simple equation for phosphate disappearance from plasma is deduced

$$y = b \cdot e^{-kt} + a \qquad (5\,c)$$

If the kinetic model and other assumptions are valid, parameters of physiological interest would be obtained by data analysis of the disappearance of phosphate from plasma after intravenous loading, since

$$k = \frac{GFR}{V_P} \text{ and } a = \frac{TmP \; 100}{GFR}$$

The relation between phosphate disappearance from plasma and excretion of phosphate is discussed on the basis of the kinetic model. Equations for the calculation of GFR, TmP and V_P using only phosphate data from clearance experiments, are deduced.

It would be of interest to test the validity of the kinetic model and the assumptions put forth in this theoretical discussion. The way in which this could be done experimentally is outlined.

MATERIAL, EXPERIMENTAL PROCEDURE AND CHEMICAL METHODS

Material

Two series of experiments (A and B) each with 11 subjects, were carried out.

Series A

The subjects were volunteers, 10 medical students aged 22—29 8 men and 2 women (none of which had been pregnant) and a fireman aged 45 All were healthy and none of them was known to have suffered from diseases of the kidneys or the urinary tracts. The fasting concentrations of plasma phosphate, determined on the morning of the experiment, varied between 2.3 and 4 0 mg %

Series B

The subjects were 10 patients with renal disorder and 1 healthy fireman, who had passed a small renal calculus some years ago Eight of the subjects suffered, or had previously suffered, from renal calculus on one or both sides, 1 subject had glomerulonephritis and 2 had stenosis of one renal artery In one of these patients the renal artery had been ligated In 10 of the subjects the 24 hours creatinine clearance had been determined before the actual experiment. It varied between 158 and 42 ml/min. The fasting concentrations of plasma phosphate determined on the morning of the experiment were normal, except in the patient with glomerulonephritis, who had a concentration of 0 1 mg %

Comments

The main purpose of these experiments was to find out whether the phosphate disappearance from the plasma in man could be described by equations, similar to those deduced from the kinetic model (Chapter I) Therefore, a series of normal subjects (Series A) was examined with frequent determinations of plasma phosphate during a long period after phosphate loading In this way data were obtained, suitable for deduction of empirical equations describing the disappearance process. If these equations agree with those set up for the kinetic model it would be possible to judge whether the constants found were of a reasonable order of magnitude.

Ten patients in Series B were treated at the hospital for various renal disorders and an evaluation of their renal function was desirable for clinical reasons. Within the series there were

patients with varying degree of renal injury and in several cases the clinical observations indicated that both glomeruli and tubules had been affected. Many of them had passed or still had renal stones Thus these patients, several of which had concrements in one or both pelvises with or without hydronephrosis, should represent the type of patient in wich renal function tests may be difficult to carry out for methodological reasons The healthy fireman in Series B originally volonteered to serve as a control, but since he had suffered from renal calculus, he was included among the patients.

Experimental procedure

Series A

The experiments started at about 8 o clock in the morning The subjects had not eaten for 12 hours and were lying down during the experiment Before the experiment they drank 1 litre of water They were allowed to drink freely during the experiment

After local anaesthesia with a small amount of 0.5 p c. lidocain, a cannula (Cournand type) was inserted in the brachial artery of one arm. A blood sample for determining plasma phosphate was taken (fasting value) In the other arm an intravenous drop of phosphate buffer solution was given.

A sterile infusion solution of the following composition was used

$Na_2HPO_4, 2H_2O$		17.514 g
$NaH_2PO_4, 2H_2O$		4 795 g
Aq dest	ad	1 000 ml

The infusion solution was isotonic and neutral. It contained 4 mg phosphate phosphorus per ml. The solution was prepared by the hospital dispensary and was supplied in 1 000 ml bottles for connecting to an infusion aggregate of the type used in the clinic.

About 1500 mg phosphate phosphorus per sq.m. body surface area was infused during about 40 minutes.

Blood samples were collected in heparinized test tubes from the arterial cannula (collection time 15—30 seconds) with 10—15 minutes interval until 180 or 240 minutes after the end of the infusion.

Series B

The experiments started at about 8 o clock in the morning The subjects had not eaten for 12 hours but had been allowed to drink water freely They were lying down during the experiment. A Foley catheter was inserted in the bladder and urine was collected to be used as urine blank for the inulin determinations. Polythene tubes were inserted in the brachial artery and the subclavian vein (from the cubital vein) using the percutaneous technique described by BERNÉUS *et al* (1054) Blood samples were taken from the arterial catheter for determination of the fasting concentration of plasma phosphate and for use as plasma blank in the inulin determinations. Samples for serum calcium determinations were sometimes taken. The subjects were allowed to drink 100—200 ml of water every 20 minutes.

Phosphate was given in the same way and in the same amounts as in

TABLE 1 *Series A Some data about the healthy subjects and the experiments*

Subject	Initials	Sex	Age	B. S. A.	Plasma phosphate Fasting value	Phosphate loading		
						Infusion time	Infused amount	
							Total	mg/sq. m. B. S. A
No.			years	sq. m.	mg %	min	mg	
A 1	R. B.	M	29	1.85	2.8	34	3104	1680
A 2	P. W.	M	22	1.79	2.3	40	2688	1500
A 3	L. G. L.	M	22	1.98	3.3	47	2904	1467
A 4	H. O.	M	21	1.80	4.0	32	3012	1673
A 5	N. F.	M	27	2.08	3.8	59	3360	1615
A 6	R. S.	M	29	1.84	4.2	47	2868	1559
A 7	U. R.	M	22	1.90	3.3	30	2800	1474
A 8	N. A.	M	45	1.88	2.6	30	2800	1489
A 9	E. R.	F	22	1.62	4.0	30	2852	1760
A 10	K. P.	F	23	1.70	3.2	30	1980	1163
A 11	G. B.	M	24	1.82	3.0	40	2800	1538

Series A except for patients B 5 and B 10 who only received half the amount. The infusion lasted from 22 to 40 minutes.

From 3 to 69 minutes after the infusion of phosphate had been discontinued a priming injection of inulin (Laevosan Gesellschaft, 10 p.c.) was given, followed by a sustaining infusion for at least 60 minutes before the collecting of urine started. For this inulin infusion, a motor-driven syringe was used. The amounts of inulin in the priming injection (total volume 100 ml) and the infusion (1 ml/min) were so calculated, on the basis of body size and available information about the kidney function, that the plasma concentration should reach about 25 mg % and remain there. No untoward reactions occurred during the inulin infusion.

The end of the phosphate infusion represents zero time. All times are given in whole minutes and were measured by a stop-watch, which was kept running throughout the experiment. Between 120 and 190 minutes urine was collected during 5—6 periods. Two to three minutes before the scheduled end of the collection period the bladder was emptied by manual compression. It was twice filled with 50 ml sterile water and emptied and then twice inflated with about 50 ml air and emptied. The moment when the last air portion left the bladder represents the end of the period. The volume of urine plus rinsing water was measured and the diuresis calculated. All urine samples were diluted first to a total volume of 400 ml for determining the phosphate concentration and then to 800 ml for determining the inulin concentration.

For plasma phosphate and inulin

TABLE 2. *Series B Some data about the patients and the experiments*

Patient No.	Initials	Sex	Age years	B. S. A. sq. m.	Plasma phosphate Fasting level mg %	Creatinine clearance ml/min	Phosphate loading: Infusion time ml	Infused amount: Total mg	Infused amount: mg/sq. m. B. S. A.	Insulin infusion started ml	Clinical diagnosis as indicated in the case record
B 1	L. D.	M	31	1.79	1.9	188	30	2685	1500	38	Nephrolithiasis l. +Susp hyperparathyreoidismus
B 2	K. J	M	38	1.94	2.5	158	30	2910	1500	10	Nephrolithiasis bil t. + Susp. hyperparathyreoidismus
B 3	G. H	M	49	2.20	3.3	—	30	3300	1500	18	Voluntee
B 4	E. I	M	45	1.98	2.7	102	30	2970	1500	69	Nephrolithiasis bilat
B 5	F. H	M	60	1.80	2.1	49	30	1400	778	60	Hypertonia + Stenosis art. ren bilat.
B 6	G. F	F	37	1.58	3.1	80	40	2400	1519	16	Hydronephosis et nephrolithiasis bil t.
B 7	O. S.	M	44	2.18	1.8	108	24	3200	1468	22	Stenosis rt. re all dx. op. (ligatur)
B 8	I. A	F	25	1.47	2.5	42	30	2200	1497	5	Nephrolithiasis sin
B 9	T. N	F	55	1.47	4.1	65	24	2200	1496	10	Ureterolithiasis et nephrolithiasis bil t.
B 10	S. P	F	21	1.73	6.1	48	20	1300	751	3	Glomerulonephritis cum syndrom nephrotico
B 11	G. A	F	55	1.58	1.7	93	23	2370	1500	5	Nephrolithiasis dx. + Susp. hyperparathyreoidismus

after end of phosphate infusion.

determinations blood samples were taken every 15 minutes up to 90 minutes and then every 10 minutes until the end of the experiment The blood was collected in heparinized tubes (collecting time 15—30 seconds) and centrifuged immediately Samples for determining serum calcium concentrations were taken 3—4 times in some of the experiments.

Comments

Pilot experiments were required to find out a mode of administration and a dosage of phosphate which did not cause untoward reactions and which gave suitable plasma phosphate concentrations.

Several investigators have shown that phosphate solutions can be given intravenously to man as single injection or infusion. A high concentration of phosphate in plasma can be tolerated over a fairly long period without discomfort. It has, however been reported (LONGSON *et al.*, 1956) that intravenous injection of concentrated phosphate solutions may cause thrombophlebitis. To avoid this, phosphate was given as short lasting infusions of isotonic solutions With this technique thrombophlebitis has never occurred Neither were other untoward reactions seen, except for sneezing and feeling of congestion in the nose at the end of the infusion period This discomfort was mild and disappeared rapidly THOMPSON & HIATT (1957) observed that serum calcium decreases after phosphate loading. A moderate fall in serum calcium with return to normal levels within 60 minutes after the end of infusion, was also observed in a few cases in the present experiments. This may possibly be the cause of the nasal symptoms. Symptoms of increased neuromuscular excitability were not seen.

Previous knowledge of the renal handling of phosphate suggests that it would be desirable to obtain a concentration fall from 10 to 5 mg % during the final part of the observation period, since at these concentration levels the tubular reabsorption of phosphate is considered to be at its maximum (Tm) (LAMBERT KESSEL & LEPLAT 1947 MCCRORY *et al.*, 1952, JACOBS & VERBANCK, 1953 LONGSON *et al* 1956 THOMPSON & HIATT 1957 LEWIS & FORD 1961 among others) In the pilot experiments it was found that a supply of about 1500 mg phosphate phosphorus per sq m. body surface area, infused over a period of 20—30 minutes in normal persons, gave a plasma concentration of inorganic phosphate amounting to 15—20 mg % at the end of the infusion The concentration 60 minutes later was about 10 mg % and after 180—240 minutes about 5 mg %

Chemical methods

Determination of inorganic phosphate in plasma and urine

For determining inorganic phosphate in plasma and urine the method described by GOMORI (1941) was used, as modified by the Department of Clinical Chemistry Lasarettet, Lund. This method is based on the fact that phosphate ions form a complex with

molybdate ions, which renders the molybdate easily reducible At the reduction a blue colour appears, the intensity of which is measured photometrically Paramethylamino-phenol sulfate in a solution of sodium bisulfite is used as reducing agent.

Reagent and material

All reagents were of analytical grade.

1 *Trichloroacetic acid* (distilled) 5 p c., in glass-distilled water
2. *Molybdate reagent* 5 g sodium molybdate p.a. (Na_2MoO_4, 2 H_2O) was dissolved in about 400 ml glass-distilled water 500 ml 1 N H_2SO_4 was added and the mixture diluted to 1000 ml (1 N H_2SO_4 29 ml concentrated H_2SO_4 [sp.gr 1.84] was added to about 100 ml distilled water and diluted to 1000 ml) The molybdate reagent and the sulfuric acid were freshly prepared each week.
3 *Para-methylaminophenolsulfate solution* 1 g paramethylaminophenol sulfate was dissolved in 100 ml 3 p c. sodium bisulfite ($NaHSO_3$) The solution was kept in a refrigerator and freshly prepared each month.
4 *Filter paper* Munktell No 3 7 cm diameter

Method

Plasma In all blood samples, the plasma was separated from the cells by centrifugation within 30 minutes after collecting the samples. Samples di playing hemolysis were discarded. To 1 ml plasma, 5 ml 5 p.c. trichloroacetic acid was added under agitation The mixture was filtered after 5 minutes. In a test tube of large diameter (Hagedorn tube) 2 ml filtrate was mixed with 5 ml molybdate reagent after which 0.25 ml paramethylaminophenolsulfate was added. (By using wide test tubes a rapid mixing with the reagent can be obtained without turning the tubes upside down.)

The colour intensity of the mixture was read in a spectrophotometer (Beckman B model) after a minimum of 45 minutes, but within 90 minutes, using a 1 cm cuvette and a wavelength of 720 mμ

Urine In order to dissolve possible phosphate sediments the urine was acidified by adding 1 ml concentrated acetic acid to each 100 ml urine It was then diluted as required for the test. The diluted urine was analysed in the same way as the plasma.

Calculation

As the reagents had a constant composition, the concentration of phosphate phosphorus in mg % of the samples can be calculated with the aid of a constant factor

The extinction 17.9 = the plasma concentration in mg %

The extinction the dilution figure 17 9 = urine concentration in mg %

Method control

The reliability of the method was continuously checked by analyses of control solutions and unknown plasma samples.

Recovery

Studies of recovery were made by analysis of mixtures of equal amounts of a phosphate solution containing

1.9 mg % phosphate and plasma with varying proportions of phosphate. Recovery averaged 96 6 p c.

Statistical evaluation of the method

The random error of the method (ε) was determined from duplicate analyses according to the formula (DAHLBERG, 1948)

$$\varepsilon = \pm \sqrt{\frac{S\,(d^2)}{2\,n}}$$

d = difference between duplicate determinations in per cent of the mean.
$S(d^2)$ = the sum of all d^2's
n = number of duplicate determinations.

For the calculation, 10 groups were selected, each consisting of 10 consecutive double determinations distributed over the concentration range 1.8—19 7 mg % (Table 3)

TABLE 3. *Determination of inorganic phosphate The random error of the method*

Range of plasma concentration mg %	Random error of the method p. c.
1.8— 3.5	1.19
3 6— 5.3	0.80
5 4— 7.1	0 98
7.2— 8 9	0.50
9 0—10.6	0.41
10 7—12.4	0 43
12.5—14.2	0 48
14.3—16.0	0.59
16.1—17.8	0 79
17.9—19 7	0 72

M = 0.68 p c. n = 100

Determination of inulin in plasma and urine

The method described by SCHREINER (1950) was used in a slightly modified form.

Inulin in protein free filtrates of plasma and urine was treated with 30 p c. hydrochloric acid and kept at a temperature of 80 for 25 minutes Thereby the inulin splits into fructose which with resorcinol in alcohol solution gives a yellow red colour the intensity of which was measured photometrically

Reagents

All reagents were of analytical grade

1 *Zinc sulfate reagent* (12 5 g $ZnSO_4$ $7\,H_2O$ + 125 ml 0.25 N H_2SO_4 + glass distilled water ad 1000 ml.)
2 *0 75 N sodium hydroxide*
3 *Resorcinol solution.* 0 1 resorcinol was dissolved in 100 ml 95 p.c. alcohol. (Freshly prepared each day)
4 *30 p c. hydrochloric acid*

Method

Plasma To 1 ml plasma was added 8 ml zinc sulfate solution and 1 ml sodium hydroxide. After thorough mixing the protein precipitate was spun down From the supernatant 2 ml was transferred to a Pyrex tube with a tightly fitting glass stopper (NS 19) 2 ml resorcinol solution was added, followed by 5 ml hydrochloric acid. The stoppers were set loosely in the tubes which were placed in a water bath with a temperature of 80 ± 1 The water-bath which is electrically heated, accomodates 42 tubes This means that all samples from a loading test can be hydrolyzed at the same time The tubes were heated for 25 minutes, after which they were at once cooled in running water for about 3—5

minutes. While waiting to be read, the tubes were kept in darkness at room temperature.

Urine The urine was diluted with redistilled water in order to obtain a suitable extinction level. 1 ml of the diluted urine was then treated in the same manner as the plasma samples.

Reading

The samples were read in a spectrophotometer (Beckman B model) at 485 mμ within one hour. The standard was read against a reagent blank. The plasma blank had an extinction of 0.020—0.030 read against water. Urine samples were read against a urine blank diluted in the same manner as the urine samples. As all inulin estimations were made in order to determine the plasma clearance, the real concentration of inulin in plasma and urine need not be calculated. The concentration of inulin in plasma and urine is therefore given in terms of the extinction 100. An extinction of 0.100 corresponds to approximately 10 mg % of inulin.

Statistical evaluation of the method

All analyses were carried out as double tests.

The random error of the method (ε) was determined from duplicate analyses according to the formula given on page 20 (DAHLBERG 1948).

For the calculation 4 groups were selected, each containing 10 consecutive double-determinations from different concentration ranges (see Table 4).

TABLE 4 *Determination of inulin. The random error of the method*

Range of plasma concentration of inulin mg/100 ml	Random error of the method p.c.
10.0—19.9	1.62
20.0—29.9	2.19
30.0—39.9	1.99
40.0—49.9	1.47
	M = 1.82 p.c. n = 40

CHAPTER III

METHODS FOR DATA TREATMENT IN DISAPPEARANCE STUDIES

In this paper phosphate disappearance from plasma after intravenous loading will be discussed, on the basis of two series of experiments. In these experiments the concentration of inorganic phosphate in plasma was repeatedly determined after the end of phosphate loading. The data will be analysed in an attempt to find an empirical equation for the relation between concentration and time during the disappearance process.

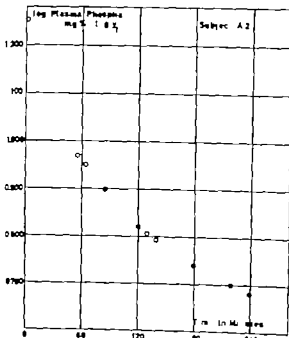
Biological disappearance processes can often, after plotting the data in semilogarithmical diagrams, be resolved into a number of exponential functions by simple graphic analysis (see e.g. RIGGS, 1963). This method for data analysis is based on the assumption that the final part of the process can be described by a single exponential function, which means that the concentration would ultimately approach zero. Therefore the method cannot be used for the analysis of phosphate disappearance from plasma since one may not assume that the plasma concentration of phosphate falls towards zero.

For this reason it was deemed suitable to determine the disappearance rate of phosphate by measuring the fall in concentration per unit of time at a large number of concentration levels and times. The relationship between the rate of disappearance and the plasma concentration was studied in order to find an empirical equation for the final part of the disappearance process. With the help of this equation the data analysis was then completed by analysis of semilogarithmical diagrams, in order to find the empirical equation for the whole of the observed process of phosphate disappearance from plasma.

Data analysis with such a purpose can be carried out in several different ways. A fairly detailed account of the technique used in this work will be given, and the different stages of the analysis will be illustrated by an example from the series of loading experiments, presented in Chapter V.

Construction of disappearance curves

The data from the loading experiments were put together in diagrams. The log plasma concentration, in mg % was plotted against time in minutes, on mm paper (285 × 340 mm, Swedish standard A3) using a scale for the concentration (y) where log 10.0 mg % =



F GURE 2. Experimental values of plasma phosphat (log y) plotted against time. The circles indicate the experimental values ± 0.0 p.c. Subject A 2, Series A.

1 000 = 500 or 1000 mm and a scale for the time (t) where 1 minute = 1 mm. The points were marked by minute Indian ink dots surrounded by pencil drawn circles.

Such semilogarithmical diagrams have the advantage that all y-coordinates will be uniformly treated when the curve is drawn. An accidental deviation of e.g. 1 mm (upwards or downwards) will give the same error calculated in percentage for any point along the curve. The units were so chosen that the main part of the curve sloped against the horizontal axis at an angle of about 45°. The measuring errors for both coordinates will then be of the same order of magnitude. This is of importance for interpolation as well as for slope analysis with the aid of an instrument.

The series of points in Fig. 2 suggest that a smooth curve could describe the phosphate disappearance from plasma. Such a curve makes it possible to study the phosphate disappearance by more advanced methods of data analysis.

Curves were drawn in pencil by hand as closely as possible to the series of dots, and in accordance with the suggestions of WORTHING & GEFFNER (1943). The curves were inked over with Indian ink using a pen of Rapidograph type and so-called Rins, that is long flexible plexiglass strips with holes for pins. By this method one can easily draw the final curve as a 0.2 mm thick, sharp line. After the pencil marks have been erased, the curve can be used for interpolation and measurements of various kinds.

The curve constructed from the data

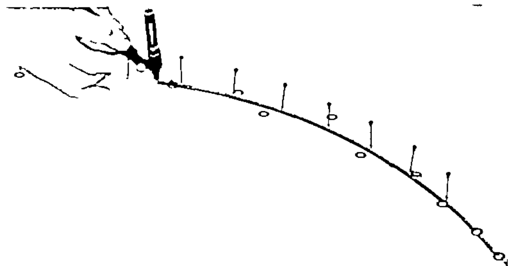


FIGURE 3. Drawing the final curve with the aid of a film.

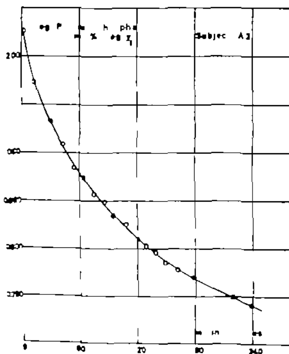


FIGURE 4. A curve constructed for the experimental values in the experiment on Subject A 2, Series A. The circles indicate experimental values ± 0 9 p.c.

of the selected experiment is shown in Fig 4

The data (plotted in Fig. 4) were marked by circles with a radius of 2 mm indicating the experimental concentration values ± 0 9 p c. It can be seen that the circles lie fairly uniformly distributed around the curve. The deviation of the concentration coordinates from corresponding points on the curve is, on an average, 0 9 p.c.. As the method for determination of phosphate in plasma has an error of 0 7 p c., the adaptation of the curve is good.

General views on slope analysis

If a disappearance process can be defined by a *straight line* in a linear coordinate system where the concentration is a function of the time, the disappearance rate is the same throughout the process and its size is given by the tangent of the angle which the line makes with the horizontal axis. As the tangent of this angle is geometrically determined by a distance on the concentration axis (mg %) divided by a distance on the time axis (min) the slope of the line or the disappearance rate in physical units is measured in mg %/min.

If a disappearance process is defined by a *curve* the disappearance rate is changing from point to point along the line The disappearance rate or the slope of the curve is then determined at every point by the sloping angle of the tangent or by the derivative (dy/dt) of the function that defines the disappearance process.

The data under discussion have been graphically represented by the function $\log y = f(t)$ where y is the plasma concentration in mg % and t is the time in minutes. Different methods for finding dy/dt for different values of y could be used.

If the plasma concentrations had been determined at equal time intervals, tabular methods for finding dy/dt might have been possible. New data can of course be extracted from the drawn curves and arranged in tabular form. This method is, however very time consuming and may provide no increased accuracy in determining dy/dt in the present investigation.

The other possibility is graphical determination of dy/dt which in its simplest form consists of the construction of tangents for the selected points of the curve and graphical determination of the slope of these tangents. From the scales, dy/dt can be calculated in physical units However a method of this kind is uncertain and time consuming It is necessary therefore to resort to some type of measuring instrument, a slope gauge or derivimeter

Slope analysis with derivimeter

ELMENDORF (1916) has devised a simple method for the construction of a normal or a tangent to a curve. If a plane mirror is placed across a curved line, the curve and its image appear as one continuous unbroken line when the mirror is exactly normal to the curve. LATHAM (1925) and SIMONS (1941) built derivimeters based on this principle SIMONS made the mirror double

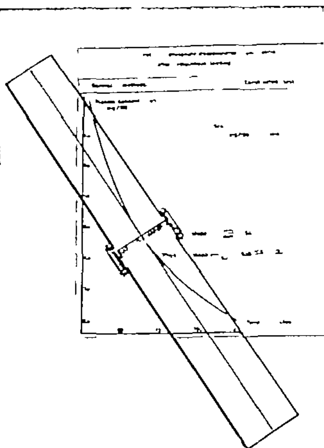


FIGURE 5 The derivimet

sided and mounted it on a plexiglass plate with a thin black line, at right angles to the mirror engraved in the bottom plane When the mirror is ad justed as described, across the curve the black line will be parallel to the true tangent at the point where the mirror intersects the curve.

HLAD & ELRICK (1959) used a me chanical derivimeter in a study of the kinetics of glucose utilization after in travenous loading

The derivimeter used in this investi gation is more or less a copy of SI MONS's instrument It consists of a 12 mm thick plexiglass plate. Its width is 90 mm and its length is 700 mm. The plate was cut in two halves with a precision cutter A plane, 0.5 mm thick, double mirror was placed between the two halves. When these were screwed together the mirror became absolutely perpendicular to the plate. A black line is engraved at right angle to the mirror The instrument is suitable for use on diagrams constructed on A3 Swedish standard paper

The point on the curve where the

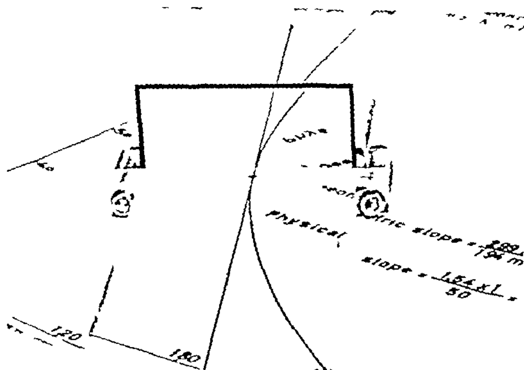


FIGURE 6 The derivimeter

slope is to be determined, is marked by a short thin pencil stroke The mirror is placed on this mark and the instrument is so adjusted that the image is a harmonious continuation of the curve The engraved line will then make a tangent to the curve at the marked point

That the instrument is correctly placed is easily checked by looking at the reflection in the other side of the mirror The work is greatly facilitated if the graph paper with the derivimeter is placed on a drawing board that can be rotated If the curve has a nick in or near the measuring point it is impossible to find a position which gives a continuous curve on both sides. The possibility of checking the position and of disclosing nicks, and the absence of movable parts give derivimeters of this kind advantages over mechanical instruments which may need to be calibrated now and then.

After the instrument has been set the geometrical slope of the curve at the measuring point is obtained by reading (in mm) from the graph the interceptions of the tangent with the two axes and dividing When the scales on the two axes are linear the physical slope (dv/dt) is obtained by multiplying the values of the geometrical slope by the scale factor

In slope analysis of curves in semi

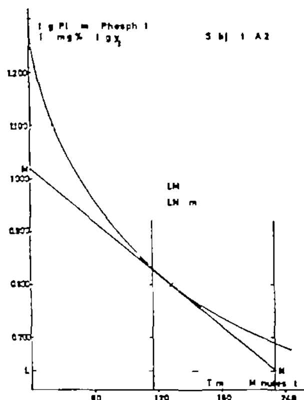


Figure 7 Determination of the geometrical slope $\left(\frac{d(\log y)}{dt}\right)$ of the curve $\log y = f(t)$

logarithmic diagrams, the recalculation with regard to the scale factors becomes somewhat more complicated The curves to be analysed are defined by the function $\log y = f(t)$ The geometrical slopes $\frac{d(\log y)}{dt}$ of the curve $\log y = f(t)$ are determined and dy/dt in physical units for corresponding points on the curve $y = f(t)$ are calculated.

The following procedure facilitates the calculations

According to the derivation rules

$$\frac{d(\log y)}{dt} = \frac{1}{y} \cdot \frac{1}{e_{\log} 10} \cdot \frac{dy}{dt} \quad (1)$$

Eq 1 can be transformed and simplified to

$$\frac{dy}{dt} = y \cdot 2.30 \cdot \frac{d(\log y)}{dt} \quad (2)$$

In order to obtain dy/dt expressed in physical units, the scale factors of the log y-axis and the t axis must be inserted into Eq 2

log y-axis 1 unit $= \beta$ mm

t axis 1 unit $= \alpha$ mm

$$\frac{dy}{dt} = y \cdot 2.30 \cdot \frac{\alpha}{\beta} \cdot \frac{d(\log y)}{dt} \quad (3)$$

From Eq 3 and Fig 7 it can be seen that it is practical to determine the geometrical slope of the curve $\log y = f(t)$ by measuring the sides of a tri

TABLE 5 *Graphic det rmination f the geometrical slope (n/m) of the curve log y=f(t) and calculation of dy/dt of th function y=f(t)*

$$\frac{dy}{dt} = y \; \frac{2.30 \;\; n}{m} \; \frac{\alpha}{\beta} \, (mg\% \; / \, min)$$

Case Subject A 2.

log y and t taken from the curve

log y = f (t)

Scale factors

log y-axis β = 10 0 mg% = 1.000 = 1000 mm.

t-axis α = 1 min = 1 mm

t min	y mg %	log y	m dm	$\frac{2.30}{m \;\beta}$	n dm	$y \; \frac{2.30}{m \;\beta} = \frac{dy}{dt}$
46	10.00	1.000	1 15	0.002	3.83—0.35	3 48 0.0200=0.070
53	9.55	0.980	1 15	0.002	3.80—0 45	3.35 191=0.064
60	9 12	0 960	1 15	0 002	3 78—0.57	3.21 182=0.058
67	8 71	0 940	1 15	0.002	3.74—0.69	3.03 174=0.053
74	8.32	0.920	1 15	0.002	3 70—0 79	2.91 166=0.048
82	7.94	0 900	1 15	0.002	3.67—0.84	2 83 159=0.045
91	7.59	0.880	1 15	0 002	3.59—0.94	2.65 152=0 040
100	7.24	0 860	1 15	0.002	3.52—1.00	2.52 145=0.037
109	6 92	0.840	1 15	0 002	3.38—1.09	2 29 138=0.032
120	6 61	0.820	1 15	0.002	3.20—1 17	2.03 132=0.027
132	6.31	0.800	1 15	0.002	3 01—1.22	1 79 126=0 023
141	6.03	0 780	1 15	0.002	1.59—0	1.59 121=0 019
161	5 75	0 760	1 15	0 002	1.55—0 12	1 43 115=0.016
178	5.50	0 740	1 15	0.002	1 46—0.20	1.26 110=0 014
197	5.25	0 720	1 15	0.002	1 42—0.27	1 15 105=0.012
218	5.01	0 700	1 15	0 002	1.39—0 29	1 10 100=0.011

angle LMN (see Fig 7) where the base is 2.30 or 1 15 dm. If the sides of the triangle are denoted by LN=m and LM=n, Eq 3 can be transformed into

$$\frac{dy}{dt} = y \; \frac{\alpha}{\beta} \; \frac{2.30 \;\; n}{m} = \frac{mg\,\%}{min} \qquad (4)$$

With the aid of vertical auxiliary lines on the diagram, placed 1 15 or .30 dm from the log y axis the values of n in dm are obtained by a single subtraction between two reading val ues. The slope should be determined at a number of points on the curve corresponding to equidistant log y-co-ordinates. The work is facilitated if the selected values of log y and of m, and the values found for n are tabulat ed as in Table 5 below The values of y are obtained from the logarithm table

Table 5 shows the results of slope analysis of the disappearance curve for the selected experiment (Subject A 2) In the diagram used for the analysis the log y axis had a larger scale unit

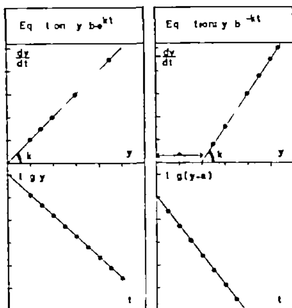


FIGURE 8. Properties of two types of exponential equations.

than that used in Figs 2, 4 and Thus the final part of the curve, which is of the greatest interest for this investigation, is straighter and could be drawn with greater accuracy

Methods for finding suitable empirical equations and for evaluating their constants

Our knowledge of the physiological processes governing phosphate disappearance from plasma after intravenous loading do not permit us to set up a rational equation for the disappearance process. A graphical method has already been described for determining dy/dt in physical units for different values of y and t after representing the experimental data by the function $y = f(t)$ where y is the plasma concentration and t the time The next stage in the data analysis is to investigate whether any definite mathematical relation can be found between dy/dt and y In some types of exponential equations, simple relations of this nature exist. In this way it may be possible to find an empirical equation that fits the experimental data from the whole process or parts of it and to determine the constants of this empirical equation.

Fig 8 shows some properties of two types of exponential equations, often seen in works on biological kinetics.

The single exponential equation $y = b\ e^{k}$ is characterized by dy/dt being a linear function of y and by the line going through the origin The slope of the line gives the numerical value of the velocity constant k. If y is expressed in mg % and t in minutes, k will have the dimension of min log y is a linear function of t The constant b can be calculated with the aid of the

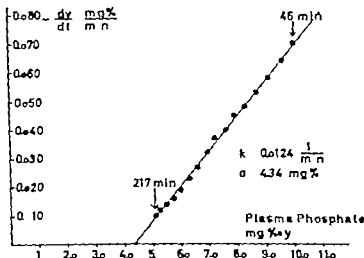


FIGURE 9 The estimated lines of dy/dt plotted against the corresponding y values. Subject A 2, Series A.

regression log y on t, graphically or mathematically because b is the numerical value of y when t = 0

For the equation $y = b \cdot e^{-kt} + a$, dy/dt is a linear function of y but the line has an interception with the y axis at y = a. A diagram where dy/dt is plotted against y thus makes it possible to determine in a simple graph, two of the three constants in the equation. The constant b can then easily be calculated if the y and t values for a point on the curve are inserted in the equation, as well as the determined k and a values, after writing the equation in logarithmic form

$$\log(y - a) = \log b - \frac{k \cdot t}{2.303} \qquad (5)$$

These methods for data analysis will be further elucidated by applying them to the results of the slope analysis of the curve from the experiment on Subject A 2. The values found for dy/dt and the corresponding y values from table 5 are shown in the diagram in Fig 9

The regression dy/dt on y can be described as a linear as −dy/dt and y in the diagram are plotted on linear scales. Phosphate disappearance from plasma in the experiment on Subject A 2 may therefore during the period 46—71 minutes and on concentration levels between 10.0 and 5.0 mg % be described by an exponential equation of the type

$$y = b \cdot e^{-kt} + a, \qquad (6)$$

where y = concentration of phosphate in plasma and t = time in minutes after the end of the infusion.

The constants k (in min⁻¹) and a (in mg %) can be calculated from the regression dy/dt on y or graphically determined. The constant b (in mg %)

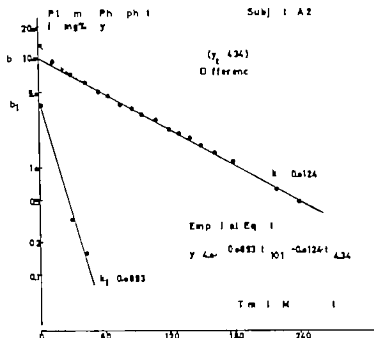


FIGURE 10. Fitting a double exponential function t the xperimental values. Subject A 2, Series A.

can be graphically determined or calculated from the regression $\log(y-a)$ on t.

The last step in the data analysis is shown in Fig 10 in which $(y-a)$ is plotted as a function of time in a semilogarithmical diagram. It may be seen that the whole of the observed disappearance process can be described by an equation (7) in which y is the sum of two exponential terms and the constant a

$$y = b_1 e^{-k_1 t} + b\,e^{-kt} + a \quad (7)$$

The constants k (in min^{-1}) and b_1 (in mg %) are determined graphically after plotting the differences against time in the semilogarithmical diagram (see Fig 10)

The first exponential term in Eq 7 is rapidly approaching zero In the example analysed this term has a value of 0 05 mg % at 56 minutes Therefore, Eq 6 will describe the further disappearance of phosphate satisfactorily

Observations concerning the accuracy of the method for data treatment

In the experiment with Subject A 2 the experimental values (y) during the final part of the disappearance process show an average deviation from the corresponding values (y_c) calculated from the empirical equations of +0.20 p c. (Table 8 Chapter V) with a standard deviation of 1 14 p c. The mean of the differences $(y-y_c)$ in p c. of y_t does not differ significantly from zero The fact that the empirical equation

gives y values well fitted to the experimental y values does not permit conclusions about the accuracy of the determination of the constants of the equation. These constants are of interest from the physiological point of view and it would therefore be useful if their accuracy were known.

It is, however, difficult to test the accuracy of the fitting of exponential functions to experimental data (FRÖBERG, 1961 RIGGS 1963) These difficulties increase with the number of exponential terms in the equation When using numerical methods the experimental data must be determined with great accuracy The phosphate concentration in plasma can only be determined with an accuracy of 2 decimals (the last one being uncertain) and already for this reason numerical methods are unsatisfactory

The suitability of the function was therefore tested in model experiments designed according to the principle of "The poor man's Monte Carlo These model-experiments were based on certain assumptions, and on the following principles

The t values of experiment A 2 were used in 6 model experiments ($l=1$ 6) and are denoted by T_j ($j=1$ 19) The errors of the t values are assumed to have a normal distribution around a mean value of zero with a standard deviation of 1 If the t values in the model experiment are called T'_{lj} we get $T'_{lj}=T_j+P_{lj}$, where P are random numbers normally distributed around 0 and with a standard deviation of 1

The function used in the model is of the principal form $f(t)=b\ e^{-kt}+a$, with the following values for the constants $b=10\ 0$ $k=0\ 01$ and $a=5.0$

The errors of the values of the function are assumed to be normally distributed around a mean value of 0 and have a standard deviation of 1 p.c. of the value corresponding to the corrected t value. Thus, if E_{lj} denotes the experimental values in the model we get

$$E_{lj}=f(T'_{lj})+Q_{lj}\ (l=1 \quad 6)\ (j=1 \quad 19)$$

where Q_{lj} are normally distributed random numbers with a mean value of 0 and a standard deviation of $0\ 01\ f(T'_{lj})$

Calculation of values (E_{lj}) for 6 model experiments, with 19 time coordinates (T_j) each, in accordance with the conditions stated above, were made with the aid of digital a computer (SMIL)

The calculated data for the model experiments are given in the appendix (Table 15)

The 6 model experiments were analysed with slope analysis of constructed curves and the constants k and a for the different experiments were calculated from the regression dE/dt on E. The results of the data analysis are shown in Table 6.

The model experiments were performed so as to be similar to experiments in Chapter V and VI They therefore allow certain conclusions about the accuracy of the constants in the empirical equations, set up to fit the actual experimental data.

The author is indebted to M Arne Sundström, B Sc., Lund, for help with this work.

Table 6. *Results of data analysis in 6 model experiments*

Model experiment No.	Values for constants in empirical equations k	
1	0.00968	4 96
2	0 01030	5 15
3	0 01008	5 06
4	0.00998	5 01
5	0.01060	5.23
6	0.00973	5 04
Mea	0.01010	5 08
S.D	0 00043	0 0985

Since the accuracy of the determination of the constant a for different values is of the same order of magnitude it was considered suitable to express the confidence limits of the constant a as an interval of 0.5 around a found value In the model experiments (see Table 6) the true value of the constant a is within the interval of found value ± 0.25

The interval for the true value of the constant k can be expressed in p c. of the found value of k, since the variation of k is dependent only on a change of angle. The model experiments show that this interval for the constant k can safely be said to be the found value ± 5 p c.

The small variations of k and a in the model experiments strongly suggest that the intervals chosen are not too narrow

Summary

In this chapter a method for data analysis is given, which is found suitable for studying disappearance processes. The method is illustrated by a detailed analysis of data from an experiment in the series reported in Chapter V The aim of the data analysis is to find empirical equations for phosphate disappearance from plasma after intravenous loading and to determine the constants of these equations.

Experimental data are plotted in a quantitative diagram and a smooth curve is constructed The slope of the curve is determined with a simple derivimeter for a number of concentration levels. The regression of the slope on the plasma concentration is first studied in a diagram. Such diagrams show whether a certain type of exponential equation can describe a disappearance process and give the constants of this equation In the selected example the final part of the disappearance process could be defined by a function which is the sum of one exponential term and one constant. After this function had been found the whole process could be resolved into two exponential and one constant term.

The accuracy of the determinations of the constants of the exponential equations was tested in model experiments performed so as to be similar to the experiments reported in Chapter V and VI. The intervals are given, within which the true values of these constants are to be found when analysing data from disappearance experiments of this type

CHAPTER IV

METHODS FOR DATA TREATMENT IN CLEARANCE EXPERIMENTS

The midpoint $t_{mp} = \frac{(t_1 + t)}{2}$ minutes, for each urine collection period, $(t - t_1)$ was determined. These values were then corrected for the renal tract delay with regard to the mean diuresis of the observation periods in each individual experiment according to Mc Swiney & de Wardener (1950). The correction was −2 minutes when the diuresis was more than 2.0 ml/min and −5 minutes when the diuresis was less than 2.0 ml/min.

The average plasma phosphate concentration ($\bar{y}$) corresponding to a urine collection period was calculated by inserting this corrected midpoint value in the empirical equation for phosphate disappearance. This equation was determined for each experiment by data analysis as described in Chapter III.

The average plasma inulin concentration ($\bar{x}$) of the period was calculated in the same way from the equation for inulin disappearance deduced by graphical analysis, i.e. the inulin concentration was plotted against time in semilogarithmic diagrams with large units on the concentration axis and the regression equation was determined for that part of the disappearance process where log x could be considered a linear function of time.

The values of the plasma phosphate concentration in the beginning (y_1) and the end (y) of a period ($t - t_1$) used for calculating the apparent volume of distribution (V_P) were determined by graphical interpolation on the disappearance curves. Also here the values were corrected for renal tract delay as described above.

For each urine collection period of the clearance experiments reported in Chapter VI the following physiological entities were thus determined using the equations deduced in Chapter I.

Glomerular filtration rate (GFR)

1) From inulin clearance

$$GFR_{In} = \frac{\overline{In_U}\ 100}{\bar{x}}\ \text{ml/min}$$

2) From phosphate data

$$GFR_P = \frac{\overline{P_U}\ 100}{\bar{y} - a}\ \text{ml/min.}$$

$\overline{In_U}$ = Mean excretion of inulin units/min

$\bar{x}$ = Average plasma inulin concentration, units/100 ml

$\overline{P_U}$ = Mean excretion of phosphate mg/min

$\bar{y}$ = Average plasma phosphate concentration, mg %

a = Constant in empirical equation for phosphate disappearance

Tubular reabsorption of phosphate (TmP)

1) From GFR_I

$$TmP_I = \frac{GFR_{In} \cdot \bar{y}}{100} - \bar{P}_U \text{ mg/min.}$$

2) From phosphate data

$$TmP_{II} = \frac{GFR_P \cdot a}{100} \text{ mg/min.}$$

Apparent volume of distribution for phosphate (V_P)

From phosphate data

$$V_P = \frac{\bar{P}_U(t - t) \cdot 100}{(y - y)} \text{ ml.}$$

y and y = Plasma phosphate concentration from graph at times t and t in mg %

$\bar{P}_U$ = Mean excretion of phosphate during the period t − t in mg/min.

Comments

Determining the average plasma concentration of a urine collection period by midpoint interpolation involves an approximation. When the concentrations at the beginning and the end of a period (t − t) are y and y_2, respectively and the disappearance is determined by a single exponential equation, the true average plasma concentration ($\bar{y}$) of the period is (WOLF 1950 SMITH, 1951)

$$\bar{y} = \frac{y_1 - y_2}{\ln y_1/y_2} = \frac{y_1 - y_2}{2.303 \cdot \log(y_1/y_2)}$$

The corresponding equation for substances the disappearance of which is determined by an equation of the type $y = b \cdot e^{-k} + a$ can easily be deduced

$$\bar{y} = \frac{y_1 - y_2}{2.303 \cdot \log\left(\frac{y_1 - a}{y_2 - a}\right)}$$

The true average plasma concentration calculated from the above equations is always higher than the corresponding concentration determined by graphical or mathematical midpoint interpolation. When the plasma concentration is falling slowly and the collection periods are short the difference is insignificant. According to WOLF (1950) it is less than 1 p.c. if the quotient y_2/y is greater than 0.61. Under the present experimental conditions there is no reason why the average plasma concentration should be determined by the more complicated method.

In a work on experimental methods like the present it seemed preferable to avoid, as much as possible, using data obtained by graphical interpolation on constructed curves. Therefore the average plasma concentrations of phosphate ($\bar{y}$) and inulin ($\bar{x}$) were calculated from empirical equations, set up for each experiment. This is easily done by inserting the corrected midpoint time value (t_{mp}) in the equations.

V_P has, however, been determined by graphical interpolation on the phosphate disappearance curves. In this way V_P for each collection period can be calculated without resorting to the empirical equation.

CHAPTER V

PHOSPHATE DISAPPEARANCE FROM PLASMA AFTER INTRAVENOUS LOADING

Series A (Healthy subjects)

Primary data from phosphate loading experiments are given in Table 16 (Appendix)

Concentration was plotted against time in semilogarithmic diagrams and disappearance curves were drawn as described in Chapter III The slopes of the curves were determined for a number of concentrations. The results are given in Figs. 11 a and 11 b, where $-dy/dt$ in mg %/min was plotted against the plasma concentration (y) in mg %

The diagrams show that, during a late phase of the disappearance process, when the plasma concentration falls from about 10 to about 5 mg % dy/dt is a linear function of y The calculated regression lines for this phase are drawn as solid lines in the diagrams The points lie close to these lines. The correlation coefficients differ only slightly from + 1 000

The investigation indicates that, during a late phase, the disappearance of phosphate from plasma can be described by an equation of the type

$$y = b\ e^{k} + a. \qquad (1)$$

The regression coefficients (k) and the y intercepts of the regression lines (a) were calculated. The constant b was determined by inserting the coordinates of one point of the curve together with the found values for k and a in the equation.

The data treatment was carried on as shown in Fig 10 Chapter III by analysis of semilogarithmic diagrams, where $(y - a)$ was plotted against time. The differences between $(y - a)$ and corresponding points on the line $(y - a) = f(t)$ were then plotted against time In every case a straight line could be fairly well adapted to this graph. Thus the whole disappearance process can be resolved into a rapid and a slow component From zero-time (the end of the phosphate infusion) the process can be described by the equation

$$y = b\ e^{k} + b\ e^{k} + a \qquad (2)$$

The constants k and b of the rapid component were determined graphically The time when the first exponential term had decreased to a value of 0 0 mg % was calculated for each experiment.

Table 7 gives the time during which the disappearance process was studied the fall in concentration and the calculated values of the constants.

The average rate constant of disappearance of the rapid phase (k) was 0 0610 min and of the slow phase (k) 0 00018 min The average of the quotient k_1/k was 0.7 which indicates that the influence of the first

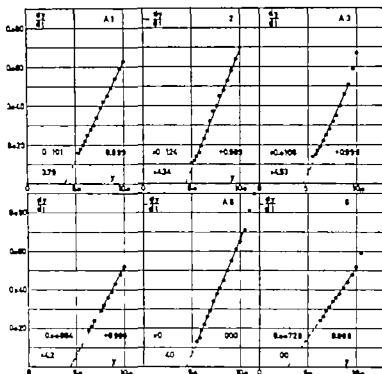


FIGURE 11 a. Series A The determined values f dy/dt plotted against the corresponding y values S bjects A 1—A 6.

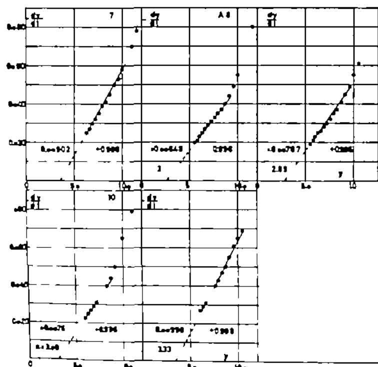


FIGURE 11 b. Series A The determined values of dy/dt plotted against the corresponding y alues. Subjects A 7—A 11

TABLE 7 *Series A. C nstants in empirical equations f r phosphate disappearance*

Subject	Range of time	Range f concentration y	Principal form of empirical equations $y = b_1 \quad + b \quad -$ Calculated values of constants					Value of t that gives $y = 0.66$ mg in equation $y = b_1 \quad - \quad t$
			b_1	k	b	k		
No.	min	mg	mg	min^{-1}	mg	min^{-}	mg	min
A 1	1—232	22.8—5.10	6 0	0 0342	13 4	0 0101	3.79	89
A 2	2—239	1 .9—4.82	4 1	0 0 39	10 1	0 0124	4.24	58
A 3	1—225	21 —3 42	6 1	0 061	11 4	0.0105	4.33	4
A 4	1—233	24 9—5 84	9 0	0 0 23	12 8	0 00354	4.21	2
A 5	1—221	20 3—3 45	3 9	0 0615	12.6	0 011	4 40	3
A 6	10—1 6	16 5—6 35	4.6	0.0692	11.9	0.00 29	2.60	66
A 7	3—210	21 6—3 55	5 1	0.0635	14.1	0 00902	3 63	0
A 8	15—210	15 8—5 51	6 3	0.0550	11.8	0 00643	2 4	84
A 9	1—240	24 2—5.44	8 2	0 0455	13 7	0 00 07	2.89	105
A 10	2—1 6	19 7—5 91	6 1	0 04 6	11 1	0.00 65	3 09	100
A 11	3—180	21 3—5 91	4 4	0.0601	14.9	0 00939	3.33	75

TABLE 8 *Series A The suitability f found mpirical equati ns f r present experi mental data from th slow phase of the disappearance process*

Time t min experimental)
Plasma phosphate y mg experimental
Plasma phosphate y_C mg (for experimental t values calculated from empirical equations)

Subject	Range of ime	Range of concentration	N mber of observations	$(y - y)$ in per cent of y	
No	t min	mg		M ± S. E. M. p c.	S. D p
A 1	93—232	8 93—3 11	11	+ 0.42 ±0 30	0.99
A 2	35—239	9 24—4 82	15	+ 0.20 ±0 29	1 14
A 3	5—225	9 32—3 42	13	− 0.20 ±0 21	0 6
A 4	79—238	10 50—5 84	13	− 0 42 ±0.33	1.2
A 5	7 —209	8 52—3 53	11	− 0.36 ±0 17	0.57
A 6	83—1 6	9 19—6 23	5	− 0 020 ±0.23	0.52
A	82—190	10.30—6 23	12	− 0 13 ±0.29	1.00
A 8	90—210	9 09 3 51	13	− 0 19 ±0.27	0.98
A 9	103—210	9 42—3 44	12	− 0 42 ±0 34	1 17
A 10	100 — 1 6	8 23—3 91	8	+ 0 058 ±0 32	0 98
A 11	80—180	10 00—3 91	11	+ 0 14 ±0 48	1.59

exponential term on the value of the function is rapidly decreasing The first term had fallen to 0 05 mg % after 78 minutes on an average and at this time the process can be considered to have entered into the slow phase, described by Eq 1

The suitability of the empirical equation to represent the data from the slow phase was tested. Values of y (y_c) for the experimental t values, were calculated from the equations. The differences ($y - y_c$) were deter mined in per cent of the correspond ing y_c-values (see Table 8)

The mean value of ($y - y_c$) was never significantly different from 0 and the average standard deviation was 0 99 p c.

Series B (Patients)

Primary data are to be found in Table 17 (Appendix)

The main purpose of the data ana lysis in this series was to find equa tions for the relation between plasma phosphate concentration and time dur ing that part of the disappearance pro cess when inulin clearance was also determined (120—180 minutes after the end of the loading) The results of the slope determinations are given in Figs. 12 a and 12 b

Also in Series B dy/dt is a linear function of y during the late phase and thus Eq 1 defines this part of the process. The constants k and a were graphically determined in this series and b was then calculated as in Series A. Also in Series B the disappearance process could be resolved into a rapid and a slow component by graphical slope analysis of the semilogarithmic diagrams The results are, however less reliable than in Series A as there are fewer data from the first 60 min

TABLE 9 *Series B Empirical equations for phosphate disappearance from plasma during the slow phase of the process*

Patient	Range of time	Range of plasma-concentration	Calculated values of constants			Empirical equations Principal form
	t	y	b	k	a	$\log (y - a) = \log b - \frac{k\ t}{2.303}$
No	min	mg %	mg %	min^{-1}	mg %	
B 1	115—195	8.75—5.48	10.2	0.00972	3.4	$\log (y-3.40) = 1.007 - 0.00422\ t$
B 2	105—195	7.24—5.16	11.7	0.00900	3.0	$\log (y-3.00) = 1.068 - 0.00391\ t$
B 3	105—185	9.79—7.16	13.2	0.00705	3.5	$\log (y-3.50) = 1.120 - 0.00306\ t$
B 4	105—195	.82—5.28	10.4	0.00726	2.7	$\log (y-2.70) = 1.018 - 0.00315\ t$
B 5	90—185	6.44—4.85	7.15	0.00444	1.7	$\log (y-1.70) = 0.854 - 0.00193\ t$
B 6	75—185	11.9—7.24	14.2	0.00780	3.9	$\log (y-3.90) = 1.154 - 0.00339\ t$
B 7	95—185	7.07—4.65	10.5	0.00787	2.2	$\log (y-2.20) = 1.020 - 0.00342\ t$
B 8	95—185	10.5—6.77	17.5	0.00878	3.2	$\log (y-3.20) = 1.243 - 0.00381\ t$
B 9	105—185	12.0—9.31	13.6	0.00510	4.0	$\log (y-4.00) = 1.134 - 0.00221\ t$
B 10	95—185	12.0—8.18	11.9	0.00423	3.9	$\log (y-3.90) = 1.075 - 0.00184\ t$
B 11	125—185	9.40—6.71	16.4	0.00724	2.6	$\log (y-2.60) = 1.216 - 0.00314\ t$

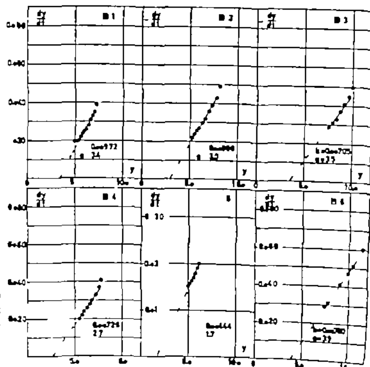
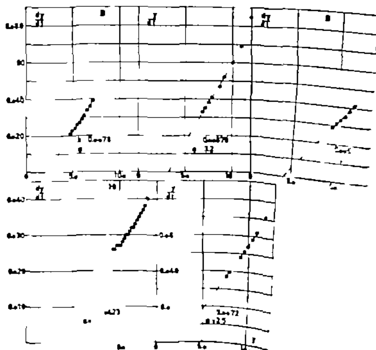


FIGURE 12 a. Series B The determined values of dy/dt plotted against the corresponding y lues. Subjects B 1—B 6.



FI URE 12 b. Series B The determined lues of dy/dt plotted against the corresponding y values. Subjects B 7—B 11

PHOSPHATE DISAPPEARANCE FROM PLASMA AND THE RENAL HANDLING OF PHOSPHATE

The clearance experiments to be discussed in this chapter were carried out on a series of patients in which the renal function was impaired to a varying degree (Series B)

Primary data about plasma inulin and phosphate are given in Table 17 (Appendix) and the corresponding values for the urine collection periods, urinary flow and excretion of inulin and phosphate in Table 18 (Appendix) In the experiment on patient B 4 two urine collection periods were combined into one period of 23 minutes, as some error of timing seemed to have occurred Otherwise all urine collection periods are recorded separately

The results of the data analysis carried out in order to obtain empirical equations for the phosphate disappearance are already given in Chapter V (Table 9)

The relation between plasma inulin concentration and time was studied as described in Chapter IV In six of the cases the inulin concentration could be considered constant throughout the experiment The mean coefficient of variation (S D in p c of the mean concentration) was 2 7 p c In the five remaining cases the plasma inulin concentration fell slightly during the experiment and the relation between concentration and time could be described by single exponential equations. The empirical equations for inulin disappearance from plasma are given in Table 11

The physiological entities GFR_P GFR_{In}, TmP_I TmP_{II} and V_P defined in Chapter IV were calculated as described in that chapter The results of these calculations are given in Table 12

The difference between the mean values of GFR_P and GFR_{In} did not in any of the experiments deviate from 0 In Fig 13 the values of GFR_P and GFR_{In} for every collection period were plotted against each other

The points of the diagram representing the 61 pairs of values lie close together along the 45 line The difference ($GFR_P - GFR_{In}$) for the whole series was $+0.000313 \pm 0.506$ ml/min (S.E.M.) and the standard deviation was 4 05 ml/min. GFR_P and GFR_I thus agree closely not only in each experiment but also in each urine collection period The glomerular filtration rate of the whole series ranged from normal figures down to 30 ml/min

Table 11 *Series B Empirical equations for the relationship between plasma inulin and time*

Patient No.	Priming injection and sustaining infusion started[1] min	Number of determinations	Range of time t min	Range of plasma concentration x_t units/100 ml	Empirical equations = f (t)
B 1	38	13	60—195	28.5 ± 0.81 (S. D)	= 28.5
B 2	10	7	60—185	12.2 — 11.3	log = 1 112 — 0 0003 t
B 3	18	9	75—185	32 5 ± 0.93 (S. D)	= 32.5
B 4	09	6	115—195	23 0 — 17 7	log = 1.518 — 0.0014 t
B 5	60	8	115—185	21 4 — 17.6	log = 1 440 — 0.0011 t
B 6	16	12	60—185	21.3 ± 0 42 (S. D)	= 21.3
B 7	22	9	105—185	21.3 ± 0 45 (S. D.)	= 21.3
B 8	5	11	75—185	26.3 ± 1 16 (S. D.)	= 26.3
B 9	10	12	60—185	43.5 — 23.0	log = 1 771 — 0.0022 t
B 10	3	10	95—185	23.0 — 20.0	log = 1 144 — 0.0003 t
B 11	5	12	60—185	32 0 ± 0 54 (S. D)	= 32.0

[1] after end of phosphate infusion

In 6 of the 11 cases (B1 3 4 6 7 and 11) the coefficient of variation (S.D in per cent of the mean) for GFR_{In} was on an average 6.4 p c. and for GFR_P 7.3 p c. In the other 5 cases the coefficient was larger than 10 p.c. when all collection periods were taken into account.

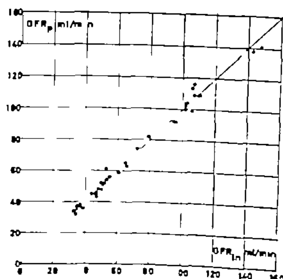


Figure 13. The relation between the values of GFR_P and GFR_{In} for 64 urine collection periods in Series B.

Patient Period No	Length of period min	Delay time min	Mid-point of period t_{mp} min	Plasma phosphate mg	mg	mg	Phosphate excreted $U_P V$ mg/min	Plasma Inulin units/100 ml	Inulin excreted $U_{In}V$ units/min	Calculated entities GFR_P ml/min	GFR_{In} ml/min	TmP_I mg/min	TmP mg/ml	V_P ml	Mean ± S.E.M. for each patient
B1 I	11	−2	124	6.43	6.45	6.31	3.16	28.5	30.3	104	106	3.68	3.54	10.9	GFR_P − 112 ± 5.5
II	10		134	6.31	6.17	6.03	3.01	28.5	31.3	109	110	3.78	3.71	11.8	GFR_I − 109 ± 2.2
III	10		144	6.03	5.91	5.81	2.61	28.5	29.1	101	102	3.42	3.54	10.9	TmP_I − 3.62 ± 0.063
IV	10		154	5.81	5.68	5.58	2.60	28.5	31.4	114	110	3.65	3.88	11.3	TmP_{II} − 3.85 ± 0.19
V	11		174	5.39	5.28	5.18	2.51	28.5	32.8	124	115	3.50	4.06	12.0	V_P − 11.3 ± 0.21
B2 I	12	−2	124	7.05	6.83	6.62	3.30	11.9	17.0	138	143	4.17	4.14	14.8	GFR_P − 121 ± 10.6
II	10		135	6.62	6.47	6.31	3.82	11.8	13.6	110	115	3.61	3.30	12.3	GFR_I − 120 ± 11.1
III	10		145	6.31	6.17	6.03	3.16	11.7	12.4	99	104	3.38	2.97	11.3	TmP_I − 3.91 ± 0.33
IV	10		155	6.03	5.90	5.75	2.46	11.6	10.2	83	88	2.73	2.35	8.79	TmP_{II} − 3.62 ± 0.32
V	10		1 4	5.52	5.44	5.30	3.69	11.5	18.0	151	157	4.85	4.53	16.0	V_P − 12.8 ± 1.05
VI	10		184	5.30	5.22	5.07	3.15	11.4	16.9	141	148	4.50	4.23	13.7	
B3 I	13	−2	125	9.23	8.98	8.73	5.02	32.5	28.8	92	89	2.93	3.22	12.6	GFR_P − 102 ± 2.3
II	9		136	8.73	8.56	8.41	5.07	32.5	33.0	100	102	3.66	3.30	14.3	GFR_I − 102 ± 2.7
III	10		145	8.41	8.24	8.09	5.00	32.5	34.4	105	106	3.73	3.68	15.8	TmP_I − 3.56 ± 0.13
IV	8		154	8.09	7.96	7.83	4.33	32.5	34.0	102	105	3.81	3.57	14.0	TmP_{II} − 3.56 ± 0.082
V	10		163	7.83	7.68	7.52	4.28	32.5	33.2	102	102	3.55	3.57	14.3	V_P − 14.4 ± 0.47
VI	10		173	7.53	7.40	7.26	4.24	32.5	34.8	109	107	3.68	3.82	15.7	
B4 I	7	−2	122	7.10	6.98	6.89	4.68	21.2	24.7	109	111	3.09	2.94	15.3	GFR_P − 109 ± 2.2
II	12		132	6.89	6. 6	6.55	4.11	21.5	20.2	103	94	2.19	2.78	14.5	GFR_I − 103 ± 2.9
III	10		142	6.55	6.40	6.28	3.91	20.8	22.2	106	107	2.91	2.86	14.5	TmP_I − 2.69 ± 0.16

TABLE 12 (continued)

B5 I	10	—5	120	5.98	5.89	5.80	1.79	20.8	9.80	43	47	0.98	0.73	9.94	GFR_P = 49 ± 4.3
II	10		130	5.89	5.71	5.63	1.47	20.2	7.96	37	30	0.76	0.63	8.64	GFR_I = 51 ± 4.2
III	9		140	5.63	5.54	5.48	2.81	19.7	13.4	66	68	1.26	1.11	15.1	TmP_I = 0.98 ± 0.080
IV	11		150	5.48	5.34	5.29	1.69	19.2	9.09	46	47	0.83	0.78	0.78	TmP_{II} = 0.84 ± 0.074
V	9		160	5.29	5.21	5.15	2.07	18.8	11.5	59	61	1.11	1.00	12.3	V_P = 11.1 ± 1.02
VI	11		170	5.15	5.06	4.98	1.52	18.3	8.44	45	46	0.81	0.77	9.81	
B6 I	11	—3	124	9.37	9.22	9.10	4.00	21.3	18.3	74	73	2.71	2.89	9.36	GFR_P = 79 ± 2.2
II	8		133	9.10	8.95	8.70	4.25	21.3	17.6	84	83	3.18	3.28	11.0	GFR_I = 78 ± 2.2
III	10		142	8.79	8.61	8.43	3.93	21.3	17.8	83	84	3.30	3.24	10.9	TmP_I = 2.97 ± 0.094
IV	11		153	8.43	8.23	8.05	3.58	21.3	16.9	82	79	3.02	3.20	10.3	TmP_{II} = 3.08 ± 0.084
V	10		163	8.03	7.89	7.75	3.83	21.3	18.2	71	71	2.77	2.77	9.43	V_P = 10.1 ± 0.30
VI	10		173	7.75	7.60	7.45	2.92	21.3	16.5	79	77	2.93	3.08	9.73	
B7 I	10	—2	123	6.34	6.17	6.04	3.83	21.3	20.7	98	97	2.16	2.11	12.7	GFR_I = 98 ± 2.8
II	10		133	6.04	5.87	5.75	3.36	21.3	20.4	92	96	2.28	2.02	11.6	GFR_I = 101 ± 2.9
III	10		143	5.75	5.60	5.50	3.29	21.3	21.7	97	102	2.42	2.13	13.2	TmP_I = 2.23 ± 0.073
IV	10		153	5.50	5.34	5.25	2.88	21.3	20.2	92	95	2.10	2.02	11.5	TmP_{II} = 2.10 ± 0.062
V	10		163	5.25	5.10	5.01	3.17	21.3	24.2	109	114	2.84	2.40	13.3	V_P = 12.6 ± 0.35
VI	10		173	5.01	4.88	4.80	2.78	21.3	21.7	104	102	2.20	2.29	13.3	
B8 I	10	5	120	9.42	9.31	8.87	3.26	26.3	14.0	54	53	1.65	1.73	6.06	GFR_I = 44 ± 4.8
II	10		130	8.87	8.60	8.41	1.90	26.3	8.60	34	33	1.00	1.09	4.13	GFR_I = 42 ± 4.8
III	10		140	8.41	8.33	7.98	1.88	26.3	9.20	37	33	1.04	1.18	4.37	TmP_I = 1.29 ± 0.14
IV	10		150	7.98	7.89	7.59	1.72	26.3	9.40	37	36	1.12	1.18	4.41	TmP_{II} = 1.40 ± 0.15
V	10		160	7.59	7.50	7.33	2.72	26.3	15.8	63	60	1.78	2.02	7.56	V_P = 8.14 ± 0.55
VI	10		170	7.33	7.14	6.89	1.50	26.3	9.60	38	37	1.14	1.33	4.41	
B9 I	10	—2	123	11.43	11.3	11.07	3.26	31.6	14.7	45	47	2.05	1.80	9.06	GFR_P = 55 ± 3.7
II	10		133	11.07	10.9	10.72	3.60	30.1	15.8	52	53	2.08	2.05	10.3	GFR_I = 55 ± 2.8
III	10		143	10.72	10.6	10.38	2.94	28.6	12.7	45	44	1.72	1.60	8.65	TmP_I = 2.23 ± 0.16
IV	10		153	10.38	10.3	10.07	3.80	27.3	15.8	61	58	2.15	2.44	12.3	TmP_{II} = 2.21 ± 0.15
V	10		163	10.07	9.94	9.77	3.76	[illegible]	17.0	63	66	2.80	2.52	12.5	V_P = 10.8 ± 0.08
VI	10		173	9.77	9.65	9.46	2.87	24.6	16.0	63	65	2.60	2.60	11.8	

TABLE 12 (continued)

1		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C10	I	10	2	153	11.12	11.0	10.81	0.93	22.2	3.32	12	15	0.72	0.51	2.00	GFR_I — 31 ± 2.7
	II	10		133	10.81	10.7	10.32	2.18	21.8	7.48	33	34	1.46	1.25	7.52	GFR_I — 33 ± 3.6
	III	10		143	10.62	10.4	10.24	2.06	21.4	8.68	32	31	1.16	1.23	7.36	TmP_I — 1.30 ± 0.16
	IV	10		133	10.24	10.1	9.96	2.26	21.0	8.12	36	39	1.68	1.40	8.07	TmP_{II} — 1.20 ± 0.14
	V	10		163	9.98	9.88	9.78	2.11	20.8	7.30	35	35	1.34	1.37	8.12	V_P — 7.04 ± 0.26
	VI	10		173	9.70	9.62	9.44	2.13	20.2	7.44	37	37	1.43	1.44	8.19	
B11	I	10	2	123	9.31	9.26	9.00	3.24	32.0	15.2	48	48	1.20	1.20	6.71	GFR_P — 50 ± 1.8
	II	10		133	9.00	8.78	8.55	3.15	32.0	16.2	50	51	1.33	1.25	7.00	GFR_I — 49 ± 1.9
	III	10		143	8.55	8.35	8.13	3.20	32.0	17.5	58	55	1.33	1.40	7.76	TmP_I — 1.17 ± 0.083
	IV	10		153	8.13	7.93	7.73	2.84	32.0	16.1	52	50	1.14	1.30	7.10	TmP_{II} — 1.26 ± 0.045
	V	10		163	7.73	7.58	7.38	2.18	32.0	13.3	43	41	0.92	1.08	6.23	V_P — 7.00 ± 0.21
	VI	10		173	7.38	7.21	7.03	2.81	32.0	16.0	53	50	1.10	1.33	7.17	

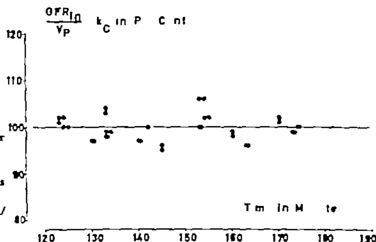


FIGURE 14. The relation between GFR_{In}/V_{P}, in per cent of the mean, and time (t_{mp}) V lues from 64 urine collection periods in Series B. Regression coefficient + 0.001 0 p c./min.

The regressions of GFR_{In} on t_{mp} (midpoint of periods) and of V_P on t_{mp} were calculated and the regression coefficients of each experiment were evaluated using the t test. In one case (B 9) GFR_{In} showed a slight tendency to rise. The coefficients of the regres sion of V_P on t_{mp} were never signifi cantly different from 0 When the regressions of GFR_{In} on t_{mp} and V_P on t_{mp} were calculated for all the 64 peri ods taken together neither of the two coefficients differed significantly from 0 GFR_{In} and V_P thus varied during the experiments but no definite tendency rising or falling could be detected.

The difference between the mean values of TmP_I (from GFR_{In} and TmP_{II} (from phosphate data) did never sig nificantly differ from 0 In the indi vidual experiments TmP_I and TmP_{II} show the same variations as GFR_{In} and GFR_P, from which they have been cal culated. The mean of the differences ($TmP_I - TmP_{II}$) for all 64 periods is $+0\ 0158 \pm 0\ 0260$ mg/min (S.E.M) and does not differ from 0 The standard deviation was 0.21 mg/min. Thus the calculation of TmP exclusively from phosphate data has proved to yield figures agreeing closely with those obtained in the classical way as the dif ference between filtered load and ex creted amount.

The quotient of GFR_{In} (in ml/min) over V_P (in ml)—corresponding to the rate constant of disappearance of the kinetic model—was determined for each collection period. Table 13 gives the mean values of the quotients (k_C) and the difference between these means and the rate constants (k) of the em pirical equations for phosphate disappearance The regression of the cal culated values of k_C (in p.c. of the mean) on t_{mp} was determined for the 11 experiments. The regression coef ficients (p c./min) with S E.M are also given in Table 13

It is obvious from the table that the values of the velocity constant, cal culated as the quotient of GFR_{In} over

TABLE 13 Series B. Comparison between rate constants found by slope analysis and rate constants calculated as the quotient (ERB_M/V_P)

Patient No.	Number of periods	Rate constants found by slope analysis (k) min⁻¹	Rate constants calculated (k_C) M ± S.D. min⁻¹	S.D. 100/M p.	$(k - k_C)$ in per cent of k p.	Regression of k_C, in per cent of the mean, on time: Coefficient of regression (B) p. /min	S.E.M. (e_B)	Significance of difference between zero and B (P)
B 1	5	0.00972	0.00937 ± 0.00040	4.1	+1.5	−0.00811	0.0531	P > 0.1
B 2	6	0.00900	0.00983 ± 0.00034	5.5	−9.2	+0.170	0.0723	0.1 > P > 0.05
B 3	6	0.00705	0.00707 ± 0.00026	3.7	−0.3	−0.0318	0.0333	P > 0.1
B 4	5	0.00726	0.00688 ± 0.00037	5.4	+5.2	−0.0805	0.138	P > 0.1
B 5	6	0.00444	0.00463 ± 0.00012	2.6	−4.3	+0.0229	0.0747	P > 0.1
B 6	6	0.00780	0.00768 ± 0.00014	1.8	+1.5	+0.0138	0.0147	P > 0.1
B 7	6	0.00787	0.00805 ± 0.00041	5.1	−2.3	+0.0666	0.128	P > 0.1
B 8	6	0.00878	0.00823 ± 0.00036	4.4	+6.3	−0.0886	0.113	P > 0.1
B 9	6	0.00310	0.00314 ± 0.00028	3.1	−0.8	+0.100	0.121	P > 0.1
B 10	6	0.00123	0.00158 ± 0.00030	6.6	−7.8	−0.151	0.163	P > 0.1
B 11	6	0.00724	0.00702 ± 0.00024	3.4	+3.0	−0.131	0.130	P > 0.1
			Mean	4.4	−0.063			
			S.E.M.	0.43	0.140			

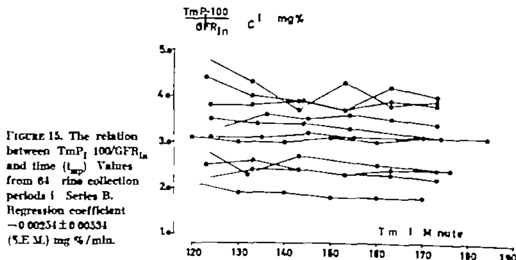


FIGURE 15. The relation between $TmP_I \cdot 100/GFR_{In}$ and time (t_{mp}). Values from 64 urine collection periods in Series B. Regression coefficient -0.00234 ± 0.00334 (S.E.M.) mg %/min.

V_F and the values obtained with slope analysis of disappearance curves are in close agreement. The differences varied between −9.2 and +0.3 p.c. The mean difference was −0.085 ± 0.149 p.c. (S.E.M.) which is not significantly different from 0. In the calculated regressions of k_C and t_{mp} the coefficient never differed significantly from 0.

In Fig. 14 k_C in p.c. of the mean was plotted against t_{mp}. The solid line of the diagram is the regression line calculated for the 64 points. The regression coefficient was +0.001 0 p.c./min and the diagram shows that the regression equation is described by a very nearly horizontal line at the 100 p.c. level.

A comparison between the values of the asymptote constant obtained with slope analysis (a) and calculated as the quotient of TmP_I over GFR_{In} (ac) is shown in Table 14.

The difference between a and ac was 0.2 mg % at most and the mean was −0.036 ± 0.039 (S.E.M.) mg % which is not significantly different from 0. The coefficient of variation for ac (in all cases) was 5 p.c. on an average. The regression of ac (in mg %) on t_{mp} was studied. In 3 cases (B 1, 5 and 11) the regression coefficient differed significantly from 0. In these experiments ac decreased with 0.46, 0.32 and 0.48 mg % respectively during 60 minutes.

In Fig. 15 the calculated values of the asymptote constant (ac) for each period were plotted against midpoint of periods (t_{mp}). The points from the individual experiments were connected by straight lines and the diagram gives a general idea of the variations of ac during the experiment. The regression coefficient of ac on t_{mp} in the whole material (n = 64) was −0.00234 ± 0.00334 (S.E.M.) mg %/min and was not significantly different from 0.

TABLE 14 Series B Comparison between asymptote constants found by slope analysis and asymptote constants calculated as the quotient TmP_I 100/GFR_I

Patient No.	Number of period	Asymptote constants found by slope analysis (a) mg %	Calculated asymptote constants (a_C) M ± S.D mg %	S.D 100/M %	Difference ($a - a_C$) mg %	Regression of a_C on t_{mp}: Coefficient of regression (B) mg % /min	S.E.M. (r_B)	Significance of difference between zero and B (P)
B 1	5	3.4	3.3 ± 0.14	4.3	+0.1	−0.0077	0.0022	P < 0.05
B 2	6	3.0	3.1 ± 0.045	1.5	−0.1	−0.00030	0.00096	P > 0.1
B 3	6	3.3	3.3 ± 0.12	3.4	0	+0.0010	0.00094	P > 0.1
B 4	5	2.7	2.9 ± 0.21	8.4	−0.2	−0.0042	0.0050	P > 0.1
B 5	6	1.7	1.9 ± 0.12	6.3	−0.2	−0.0054	0.0016	P < 0.05
B 6	6	2.9	2.8 ± 0.077	3.0	+0.1	+0.00023	0.0021	P > 0.1
B 7	6	2.2	2.3 ± 0.089	3.9	−0.1	−0.0011	0.0023	P > 0.1
B 8	6	3.2	3.1 ± 0.045	1.5	+0.1	+0.00029	0.0012	P > 0.1
B 9	6	4.0	4.0 ± 0.24	6.0	0	−0.0046	0.0040	P > 0.1
B 10	6	3.9	4.1 ± 0.41	10.0	−0.2	−0.018	0.0072	P > 0.1
B 11	6	2.5	2.4 ± 0.16	6.7	+0.1	−0.0080	0.0018	P < 0.01
			Mean	5.0	−0.036			
			S. E. M	0.83	0.039			

Discussion

The assumptions made in Chapter I when deducing the kinetic model can now be discussed in the light of the results here presented. The investigation shows that at plasma phosphate concentrations of 10 to 5 mg % the glomerular filtration rates, calculated from phosphate data only (GFR_P) and determined using inulin clearance, are practically identical, when comparing values from one period as well as when comparing mean values for each experiment. TmP calculated from inulin clearance (TmP_I) and from phosphate data only (TmP_{II}) show the same close agreement. This double correspondence does not speak in favour of the view that any essential part of the plasma phosphate should be non filtrable under the present experimental conditions.

Neither do the results support the theory that a tubular secretion dependent on the plasma concentration plays any great role for the excretion of phosphate at these concentration levels.

The variations of the calculated values of GFR, TmP and V_P from period to period may be caused by physiological variations or by technical errors for instance when collecting the urine. In Series B the aim was to keep the glomerular filtration rate as constant as possible. In about half of the cases GFR_{In} and the other entities determined varied to an extent usually seen in inulin clearance experiments performed under optimal conditions (Smith 1956, Falkheden 1963). In the other 5 cases the variations were greater. In this group the filtration rate was reduced considerably in 4 cases and it is quite possible that technical errors have affected the results particularly as the diuresis in some of the cases was low (B 5 and 8). A study of the variations of GFR_{In} and V_P during the experiment did not disclose any certain tendency rising or falling.

The quotients GFR_{In}/V_P and TmP_I/GFR_{In} were also studied. They showed no tendency to rise or fall in the material as a whole. It was further found that the mean values of these calculated quotients correspond to the values of the rate constant of disappearance and the asymptote constant obtained by slope analysis in every single experiment. This supports the assumption that these constants have the physiological significance which was ascribed to them when deducing the equation for plasma phosphate disappearance from the kinetic model. The investigation thus speaks in favour of the validity of the model and the assumptions made in connection with it.

The result of the clearance experiments make it probable that the corrections used for renal tract delay are reasonable. In several cases plasma phosphate concentration was falling slowly which makes a wrong estimate of the delay time of small importance. Even in the cases where the phosphate concentration was falling relatively rapidly and the inulin concentration was constant a close agreement between the values of GFR_{In} and GFR_P was seen. It should thus be possible

to determine GFR and TmP from plasma phosphate data with the method developed here. The same accuracy seems to be achieved as when TmP is calculated from GFR using inulin clearance.

The practical value of the findings in Chapter V can be judged by the results reported in the present chapter. In Chapter V it was shown that the disappearance of phosphate from plasma during a certain phase of the process can be described by an exponential equation with three constants, both in normal subjects and patients in which the renal function was impaired to a varying degree. The velocity and asymptote constants of this equation were found to have a physiological significance. It is thus possible to determine GFR/V_F, the velocity constant, and TmP · 100/GFR, the asymptote constant, by slope analysis of the phosphate disappearance curve after single injection or brief infusion. The results presented here and in Chapter III imply that this slope analysis is accurate enough to be acceptable in clinical work.

As there are reasons for believing that neither GFR nor TmP are absolutely constant in one and the same individual but show a co-variation (ANDERSON and PARSONS, 1963) it is possible that the quotient of these entities is of greater interest in some connections than the entities themselves. In certain disorders a determination of the quotient may give information regarding the main site of the functional disturbance and if further information is needed the absolute figures of GFR and TmP should be obtainable by analysis of the excretion of phosphate during *one* relatively long urine collection period. Probably the quotient also has the advantage of being independent of body size, which is of value when comparing different subjects.

The results of the experiments on Series B also indicate that an analysis of the disappearance curve of phosphate as presented here can be applied to clinical cases where there are reasons to suspect a disturbance of the renal handling of phosphate (for instance hyperparathyreoidism and genetic disturbances of the tubular function). This method for determining GFR, exclusively from phosphate data, may also be of use in studying the renal function in cases where it is difficult to determine the inulin concentrations (e.g. in subjects with glycosuria) or where a constant infusion of inulin is difficult to perform.

The evaluation of the usefulness of the present method in clinical work has to be postponed until observations on a larger number of patients with various diseases are available.

SUMMARY

A kinetic model representing the distribution and elimination of inorganic phosphate from plasma after intravenous loading was set up. This model is assumed to be valid for a late phase of the process of phosphate disappearance, when the tubular reabsorption is still at its maximum. From the model equations were deduced for the relation between plasma phosphate concentration and time during the relevant phase of the disappearance process. This equation is

$$y=b\ e^{k\,t}+a$$

where y denotes plasma concentration in mg % and t the time in minutes after end of loading. The constants k and a have the following physiological significance

$$k=\frac{GFR}{V_P}\,min \quad \text{(rate constant of disappearance)}$$

$$a=\frac{TmP}{GFR}\,100\ mg\ \% \quad \text{(asymptote constant)}$$

where GFR is the glomerular filtration rate in ml/min, V_P the apparent volume of distribution for phosphate in ml and TmP the maximal tubular reabsorption of phosphate in mg/min. Equations for calculating GFR and TmP when the phosphate excretion is known, were deduced from the model.

The disappearance process was investigated without using the kinetic model, and the equation deduced from it. Slope analysis of disappearance curves from loading experiments on normal subjects and patients was carried out with the aid of a derivimeter. The relation between the slope or the rate of disappearance (dy/dt) and the plasma concentration (y) was studied. The regression of dy/dt on y showed that a late phase of the disappearance process can be described by equations giving the plasma concentration as the sum of one exponential term and one constant. These equations correspond to the one deduced from the kinetic model.

As the ordinary methods for testing the fitting of experimental data to empirical equations could not be used, the accuracy of the determinations of the constants k and a was evaluated in model experiments. These were performed so as to resemble the actual experiments as closely as possible with regard to the distribution of the experimental data and the range of concentration and time. The data analysis of the model experiments proved that the constants k and a can be determined with sufficient accuracy for clinical purposes.

The disappearance of phosphate from plasma was thus studied in one

series of 11 normal subjects and one series of 11 patients in which the renal function was impaired to a varying extent. In the last group the glomerular filtration rates ranged from 126 to 31 ml/min. In the normal subjects the values of the constants k and a obtained by slope analysis were of a reasonable order of magnitude considering the physiological significance attributed to them when setting up the model. The value of k was significantly lower in the patients than in the normal subjects, whereas the constant a was of the same order in both series.

Clearance experiments were performed on the 11 patients. Inulin clearance (GFR_{In}) was determined using constant infusion technique. GFR was also calculated from phosphate data (GFR_P). Very good agreement between the values of GFR_I and GFR_P was obtained both within every single urine collection period and in the individual experiment as a whole. The values of TmP calculated in the classical way using inulin clearance and calculated from phosphate data exclusively showed the same close agreement.

The quotients GFR_{In}/V_P and TmP 100/GFR_{In} were calculated for each period. The means of these quotients were compared with the values of k and a obtained by slope analysis. No significant differences were found. The values of the quotients remained constant throughout the experiments.

The results imply that at the concentration levels used (about 10—5 mg %) inorganic phosphate in plasma may be considered entirely ultrafiltrable. Furthermore, the results do not speak in favour of the assumption that there is a tubular secretion of phosphate dependent on plasma concentration.

The analysis of the relation between phosphate disappearance from plasma and the renal handling of phosphate has thus shown that during a late phase, *the process of disappearance is in accordance with a kinetic model* deduced for the distribution and elimination of phosphate and that under certain experimental conditions *the glomerular filtration rate and the maximal tubular reabsorption may be calculated using only phosphate data*.

The value of analysis of phosphate disappearance from the plasma in clinical work is discussed.

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APPENDIX

(Tables 15—18)

TABLE 15. *Calculated data f r 6 model experiments (i=1 6)*

i	j	1	2	3	4	5	6	7	8	9
	T_j	2	13	30	43	53	64	75	86	93
1	F_{ij}	14.9	13.5	12.4	11.8	10.8	10.2	9.50	9.31	9.01
2	E_{ij}	14.8	13.9	12.3	11.5	10.6	10.3	9.68	9.18	8.83
3	C_{ij}	14.7	14.2	12.2	11.3	10.7	10.4	9.59	9.24	8.73
4	F_{ij}	14.5	14.1	12.5	11.5	10.7	10.3	9.78	9.21	8.90
5	E_{ij}	15.0	13.6	12.3	11.5	10.7	10.2	9.62	9.21	8.72
6	F_{ij}	14.8	13.7	12.4	11.3	10.7	10.4	9.79	9.29	8.74

i	j	10	11	12	13	14	15	16	17	18	19
	T_j	108	120	129	130	149	162	179	201	210	230
1	F_{ij}	8.51	7.98	7.87	7.58	7.44	7.00	6.66	6.32	6.02	5.86
2	E_{ij}	8.35	7.99	7.81	7.39	7.33	7.07	6.63	6.36	6.13	5.99
3	C_{ij}	8.36	7.93	7.75	7.31	7.11	6.96	6.60	6.27	6.06	5.97
4	F_{ij}	8.43	7.81	7.74	7.57	7.34	7.07	6.55	6.18	6.19	5.89
5	E_{ij}	8.30	8.00	7.71	7.44	7.21	6.93	6.71	6.33	6.07	5.97
6	F_{ij}	8.40	8.17	7.81	7.38	7.22	7.10	6.79	6.42	6.14	5.98

TABLE 16. *Series I. Plasma phosphate after intravenous loading*

Subject No.	Primary data: Time (t) in minutes and plasma phosphate concentration (y_1) in mg %. End of infusions at time 0 minutes																						
A 1	t	1	11	19	30	40	53	61	70	86	95	105	114	126	136	144	154	161	184	206	232		
	y	22.8	18.5	16.6	14.9	13.4	12.1	10.9	10.6	9.34	8.95	8.50	8.01	7.51	7.14	6.95	6.60	6.41	5.91	5.41	5.10		
A 2	t	2	13	30	43	55	64	75	86	95	108	120	129	139	149	162	170	204	219	239			
	y	17.9	12.9	11.8	10.4	9.31	8.91	8.22	7.86	7.39	7.09	6.55	6.37	6.10	5.93	5.69	5.50	5.22	4.90	4.85			
A 3	t	1	17	28	39	49	61	75	84	91	101	114	127	147	167	180	197	207	218	229			
	y	21.7	15.6	13.8	12.6	11.5	10.3	9.52	8.95	8.64	8.27	7.80	7.41	6.81	6.41	6.03	5.91	5.75	5.58	5.13			
A 4	t	1	12	21	32	42	53	64	79	89	100	110	122	151	160	173	183	193	202	218	228		
	y	21.9	18.9	16.9	14.9	13.2	12.4	11.2	10.5	9.07	9.43	8.95	8.50	7.01	7.37	6.75	6.59	6.37	6.32	6.00	5.84		
A 5	t	1	12	22	33	45	56	66	77	93	105	118	131	160	171	181	191	201	209	221			
	y_1	20.3	17.1	15.1	13.5	12.0	11.0	10.2	9.52	8.63	8.09	7.61	7.09	6.34	6.01	5.89	5.73	5.57	5.33	5.45			
A 6	t	10	25	10	60	82	103	131	156	176													
	y_1	16.5	13.8	12.2	10.0	9.49	8.42	7.19	6.82	6.25													
A 7	t	3	15	31	46	60	70	82	90	100	110	120	131	140	150	160	170	181	190	199	210		
	y_1	21.6	17.9	15.9	13.2	12.0	11.2	10.3	9.85	9.36	8.93	8.32	8.01	7.61	7.27	6.80	6.71	6.39	6.33	5.84	5.66		
A 8	t	15	30	45	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210			
	y	15.8	13.4	12.1	10.7	10.1	8.42	9.09	8.75	8.27	7.91	7.61	7.09	6.95	6.77	6.11	6.25	5.91	5.69	5.81			
A 9	t	1	13	22	33	41	52	61	72	83	93	103	112	123	153	163	172	183	192	203	213	227	240
	y_1	21.2	19.2	17.1	13.6	14.3	13.0	12.3	11.3	10.7	9.99	9.42	8.97	8.62	7.61	7.27	7.03	6.84	6.41	6.09	5.76	5.57	5.14
A 10	t	2	17	33	47	57	67	78	89	100	111	121	133	143	153	161	176						
	t	18.7	15.2	12.9	11.5	10.7	10.0	9.66	8.72	8.25	7.88	7.42	7.16	6.80	6.48	6.37	5.91						
A 11	t	3	19	33	47	60	70	80	90	102	110	120	136	141	150	160	172	180					
		21.5	16.9	14.8	13.1	12.0	11.0	10.0	9.02	8.93	8.43	8.11	7.00	7.18	6.90	6.63	6.25	5.91					

TABLE 17 Series B Pl ma calcium and plasma phosphate after intravenous loading

Patient	Primary data: Time (t) in minutes; Plasma calcium (c) in mg/100 ml; Plasma phosphate (y) in mg/100 ml; End of infusion of phosphate at time 0 minutes																		
H 1	t	30	60	90	98	105	115	125	135	145	155	165	175	185	195				
			27.7	30.2	29.0	29.0	28.0	28.6	27.8	28.3	28.8	28.9	28.5	27.0	27.8				
		12.0	9.79	7.81	7.25	8.78	6.75	6.46	6.07	6.91	5.85	5.45	5.31	4.98	4.37				
H 2	t	10	17	32	45	60	75	90	95	105	115	125	135	145	155	165	175	185	195
						12.2		12.2		13.2		13.0		11.9		11.2		11.2	
		16.6	13.3	13.0	11.5	10.0	8.77	8.77	8.11	7.34	7.35	6.77	6.48	6.00	5.80	5.58	5.37	5.01	5.16
H 3	t	15	30	45	60	75	90	95	105	115	125	135	145	155	165	175	185		
			31.0	37.0	35.7	33.5	31.0	33.3	31.7	32.5	31.4	32.0	32.4	32.6	32.3	33.0	30.7		
		19.8	16.0	14.0	12.7	11.5	10.7	10.5	9.79	9.49	9.18	8.58	8.28	7.95	7.61	7.37	7.16		
H 4	t	16	30	45	60	75	90	95	105	115	125	135	145	155	165	175	185	195	
						40.8	31.4	29.0	33.3	33.0	31.9	32.1	30.4	19.5	19.0	19.0	20.9	17.7	
		14.9	13.1	11.8	10.2	8.05	8.33	7.97	7.32	7.18	6.89	6.62	6.44	6.09	5.85	5.61	5.55	5.29	
H 5	t	15	30	45	62	75	90	95	105	115	125	135	145	155	165	175	185		
					32.3	31.5	31.5	31.0	33.0	21.4	22.4	20.0	19.3	18.5	18.7	18.2	17.5		
	y	9.03	8.68	7.97	7.16	6.98	8.14	8.35	8.23	6.00	5.78	5.64	5.29	5.37	5.12	4.09	4.65		
H 6	t	15	30	45	60	75	85	104	115	125	134	145	155	165	175	185			
					31.0	20.7	31.0	31.3	31.3	31.6	31.6	20.9	21.1	20.9	22.5	31.6			
	y_t	18.3	16.0	14.1	12.8	11.9	10.8	10.2	9.67	9.49	8.85	8.50	8.20	7.84	7.52	7.34			
H 7	t	15	30	45	60	78	95	105	115	125	135	145	145	165	175	185			
					31.2	23.0	22.3	22.0	21.1	31.3	20.8	21.2	21.4	21.2	22.1	20.9			
	y_t	15.5	11.9	9.90	8.77	7.70	7.07	6.86	6.44	6.27	5.91	5.55	5.37	5.07	4.80	4.83			
H 8	t	15	30	45	60	75	95	103	115	125	137	145	155	165	175	185			
						23.3	27.2	26.8	25.6	25.5	25.0	25.0	26.0	25.5	27.5	27.0			
	y_t	19.7	17.3	15.2	13.9	12.4	10.5	10.3	9.83	9.31	8.41	7.97	7.52	7.29	6.86	6.77			
H 9	t	16	30	45	60	75	93	103	115	125	135	145	155	165	175	183			
		76.3	50.3	49.0	43.3	40.0	36.5	34.5	33.0	30.9	30.0	28.0	27.0	26.0	24.0	23.8			
	t	18.5	18.6	13.4	14.4	13.3	12.2	12.0	11.8	11.1	10.7	10.8	9.99	9.85	9.26	9.21			
H 10	t	15	30	45	60	75	85	103	118	125	135	145	155	165	175	185			
					26.0	25.5	22.0	21.0	22.0	21.8	21.8	20.8	21.0	20.8	20.0	20.0			
	t	13.5	14.2	14.0	13.3	12.8	12.0	11.4	11.2	11.1	10.6	10.1	10.1	9.03	9.19	8.18			
	t	15	30	45	60	75	95	105	115	125	125	145	155	165	175	185			

TABLE 18 *Series B Excretion of inulin and phosphate during urine collection periods in clearance experiments*

Primary data. Time (t) in minutes. Urine flow (V) in ml/min. Excretion of inulin ($\overline{In}_C$) in units/min. Excretion of phosphate ($\overline{P}_C$) in mg/min. End of infusions of phosphate at time 0 min [illegible]

Patient No.	Period	I	II	III	IV	V	VI
B 1	t	120 — 131	131 — 141	141 — 151	151 — 161	1 0 — 181	
	V	4.3	3.2	2 9	2.4	3.2	
	$\overline{In}_C$	30.3	31.3	29 1	31 4	32.8	
	P_C	3 16	3.01	2.61	2 60	2.51	
B 2	t	120 — 132	132 — 142	142 — 152	152 — 162	171 — 181	181 — 191
	V	—	—	—	—	—	—
	$\overline{In}_C$	17.0	13.6	12.4	10 2	18 0	16.9
	P	5 30	3 83	3.13	2.46	3.69	3.15
B 3	t	120 — 133	133 — 142	142 — 152	152 — 160	160 — 170	170 — 180
	V	—	—	—	—	—	—
	$\overline{In}_C$	28.6	33.0	34 4	34 0	33.3	34 8
	P	5 02	4.07	5 00	4 85	4 28	4.24
B 4	t	121 — 128	128 — 140	140 — 150	150 — 173	173 — 183	
	V	2.9	1 7	3 0	2 9	5.5	
	$\overline{In}_C$	24 7	20.3	22 2	20.9	20 8	
	P_C	4.06	4 11	3.91	3.69	3 39	
B 5	t	120 — 130	130 — 140	140 — 149	149 — 160	160 — 169	169 — 180
	V	1 1	0.7	0.9	0.3	0 7	0 6
	$\overline{In}$	9.80	7 96	13 4	9.09	11.5	8.44
	P	1 79	1 47	2 31	1.69	2 07	1 53
B 6	t	120 — 131	131 — 139	139 — 149	149 — 160	160 — 170	170 — 180
	V	8 4	8 1	7 4	8 2	8 8	7.8
	$\overline{In}_C$	15.3	17 8	1 8	16.9	15 2	16.5
	P	4 00	4 25	3 93	3.36	2 83	2 92
B 7	t	120 — 130	130 — 140	140 — 150	150 — 160	160 — 170	170 — 180
	V	3 2	3 1	3.3	2 8	3 3	3.6
	$\overline{In}_C$	20 7	20 4	21 7	20 2	24 2	21 7
	P_C	3 82	3 36	3 20	2 83	3.17	2.78
B 8	t	120 — 130	130 — 140	140 — 150	150 — 160	160 — 170	170 — 180
	V	1.8	1 1	0 6	0.4	1 0	0.5
	$\overline{In}_C$	14.0	8 80	9.20	9 40	15 8	9 60
	P	3.28	1.90	1.88	1 72	2.72	1.50

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PREFACE

In 1952 Waldenström described purpura hyperglobulinemica in a 32 year-old female and added the case report of her dizygotic twin sister who had a chronically high erythrocyte sedimentation rate and cutaneous manifestations consistent with lupus erythematosus. Later the former patient developed classic systemic lupus erythematosus. Waldenström foresaw the importance of genetic factors in these syndromes. He suggested an investigation of the family in question, the results of which were published in 1957 and in 1959 (Leonhardt 1957, Larsson and Leonhardt 1959). The present investigation, which was carried out under the guidance of Professor Jan Waldenström was undertaken in an attempt to elucidate further the role played by heredity in the causation of systemic lupus erythematosus.

I wish to express my thanks to Professor Jan Waldenström for discussions on the concept of systemic lupus erythematosus and allied collagen and autoimmune syndromes, for fruitful suggestions, and for placing the facilities of the Department of Internal Diseases, Malmö General Hospital, at my disposal.

Acknowledgement is due to Professors C-G Ahlström and F Lindell for examining biopsy and necropsy specimens, Professor S Winblad for the performance of most of the serologic tests at his laboratory and for facilities for the preparation of fluorescent antihuman serum; Dr B Hederstedt, *Statens Bakteriologiska Laboratorium* for performing the treponema pallidum immobilization test on some of the sera, Dr B Willert for help with the ABO and Rh grouping of probands' sera and for placing test sera at my disposal, Professor E Mandema, Groningen and Dr W Hijmans, Leiden, The Netherlands, for instruction and advice on the fluorescence technique for demonstrating antinuclear factors; Associate Professor C-B Laurell for advice on the paper electrophoretic technique for study of serum proteins and for immunoelectrophoretic analysis of the fluorescent antihuman serum, Mrs I Falk for technical assistance with the electrophoresis and the preparations for demonstration of antinuclear factors; the staff of the Department of Bacteriology for performing the serologic tests; Professor C-E Quensel for advice on statistic methods, Dr C-A Larsson for advice on genetic and genealogic questions; Mrs I Bräutigam-Ericson for genealogic archives studies; Professor G Edström, Professor N Söderström, Dr Å Bergwall and Dr H Lindholm for kind permission to publish some of their cases; all the chiefs of the departments and laboratories of hospitals in Scania for kind cooperation in the search of their archives for cases of systemic lupus erythematosus.

The investigation was supported by grants from *Lunds Universitet*, *Konung Gustaf V:s 80-årsfond*, *Ernhold Lundströms stiftelse*, *Alfred Österlunds fond*.

Parts of the investigation were presented at the VIIth International Congress of Internal Medicine in München 1962 and at the Vth European Congress on Rheumatic Diseases in Stockholm 1963.

RFs — rheumatoid factors
SSC-factors — the serum protein factors responsible for reactions in the Heterophile Absorbed Sheep Cell Test (SSC-test)
FII AP factors — the serum protein factors responsible for reactions in the FII Acryl Particle Fixation Test (FII AP-test)
FIIA SC factors — the serum protein factors responsible for reactions in the FIIA Coated Tanned Sheep Cell Test (FIIA SC-test).
ANF — antinuclear factors
STS-factors — the serum protein factors responsible for reactions in the serologic tests for syphilis (STS)
ASL — antistreptolysin 0

The strength of the serologic reactions was expressed as the reciprocal of the lowest dilution (titre) giving visible reaction in the test tubes. The titres of ASL were given in conventional units (*U*)

Other laboratory terms

ECG — electrocardiography
ESR — erythrocyte sedimentation rate (Westergren)
NPN — non-protein nitrogen of the serum
TPI-test — treponema pallidum immobilisation test
RBC — red blood cell count
WBC — white blood cell count

Statistic terms

Mean — arithmetic mean
P — probability
SD — standard deviation
SE — standard error of mean

REVIEW OF LITERATURE

HISTORIC DEVELOPMENT

In the following historic review stress has been laid upon the changes undergone from time to time by the general concept of SLE. Table I gives a summary with special reference to the nomenclature. Reviews of the history of SLE have been given by Harvey *et al* (1954), Talbott and Ferrandis (1956), Hill (1957) and Muehrcke *et al* (1957).

It appears that in the beginning of the 19th century no distinction was made between the *cutaneous* and *systemic* forms of lupus erythematosus: the skin lesions were described without any attempt to relate them to other symptoms. Towards the end of the century, however, descriptions of cases with systemic manifestations and even with a fatal course began to appear (Kaposi 1872). Far into the 20th century SLE was not always clearly distinguished from other diseases with cutaneous manifestations and was commonly thought to be a variant of tuberculosis. Even typical cases of SLE were reported under a wide variety of names (see e.g. Helfenstein *et al* 1939). It was not until the 1920s and 1930s that SLE was described as a clinical entity (see e.g. Goeckerman 1923). The concept of SLE as a distinct disease crystallised largely from the contributions of pathologists, who described morbid-anatomic changes that were soon accepted as characteristic of SLE: the atypical verrucous endocarditis reported by Libman and Sacks (1921), the wire-loop lesions and other findings detailed by Baehr, Klemperer and Schifrin (1935). A fairly clear notion of SLE emerged: an acute exanthematous disease with predilection for young females, with a poor prognosis, and with pathognomonic lesions of the inner organs.

Neumann (1880) was the first to use the term "fibrinoid degeneration" to designate certain alterations in the structures of the connective tissue and Klinge (1933) pointed out the widespread occurrence of such alterations in the rheumatic diseases. Klemperer, Pollak and Baehr (1942) introduced the term "diffuse collagen diseases" to emphasize the generalized involvement of the connective tissue in SLE as well as in some other diseases, particularly rheumatic fever, rheumatoid arthritis, scleroderma, dermatomyositis and polyarteritis nodosa. Clinicians (Banks 1941 and others) had already begun to recognize the clinical similarities of these syndromes and soon adopted the term "collagen disease" as a common denomination. The successful therapeutic use of ACTH and corticosteroids in these conditions, prompted by Hench's demonstration of the beneficial effect of cortisone on rheumatoid arthritis (1949), strengthened the consensus that the collagen diseases were closely interrelated, and the definition of SLE again tended to become diffuse.

The discovery by Hargraves, Richmond and Morton (1948) of the LE-cell phenomenon gave rise to a surge of interest in SLE. The phenomenon soon became the most important diagnostic adjunct in SLE and was thought to be specific. This resulted in further broadening of the concept of SLE: cases without typical skin

Table 1. Historic remarks, with emphasis on nomenclature

1851	Cazenave is said t have been the first to use the term "lupus erythematosus
1856	Hebra distinguished between local and widespread lupus erythematosus
1872	Kaposi introduced the term "lupus erythematosus disseminatus and reported cases with generalized symptoms. First description of *systemic* lupus erythematosus?
1877	Neumann depicted the histologic changes in cutaneous LE and was the first t apply the term "fibrinoid degeneration
1924	Libman and Sacks presented their classic description of "atypical verrucous endocarditis
1935	Baehr Klemperer and Schifrin gave a detailed account of the morbid-anatomic changes in SLE, including the so-called "wire-loop lesions
1937	Keil coined the term "systemic lupus erythematosus
1942	Klemperer Pollack and Baehr created the term diffuse collagen disease thereby arousing interest in the generalized involvement of *connective tissue* in SLE and in related syndromes
1948	Hargraves *et al* discovered the "LE-cell phenomenon ushering in an epoch of intense research on the *immunology* of SLE
1949	Hench *et al* demonstrated th beneficial ffect of cortisone on rheumatoid arthritis which resulted also in dvances in the treatment of SLE and more optimistic view on its prognosis
1949	Burnet *et al* presented theories on the mechanism of immunity ("clonal selection"), and in 1953 and later Medawar *et al* ga an experimental basis for "immunologic tolerance which has contributed substantially to our present concept of "autoimmune diseases
1951	Davis and Gutridge published the first adequat description of "familial SLE
1958	Dameshek summed up the new aspects on SLE by calling it "a complex autoimmune disorder"

lesions and without histologic proof of the diagnosis were accepted as SLE as long as they showed a positive LE-cell test. The frequent finding of a positive LE-cell phenomenon also in RA and in the other collagen diseases tended to blur the limits still more The roughly simultaneous introduction f corticoster oid therapy contributed also to another view on the *prognosis* of SLE. This revision of the concept of SLE is clearly reflected in the extensive surveys by Dubois (1953) and Harvey *et al* (1954) who stress both the diagnostic importance of the LE-cell phenomenon and the frequent chronicity f SLE.

The discovery f the LE-cell phenomenon also prompted an intense search for blood protein abnormalities in SLE. Patients with this syndrome were almost in variably found to have not only hyper gammaglobulinemia but also a variety of gammaglobulins behavi g serologically like antibodies to normal components of the bod —autoantibodies. Th general concept of autoimmunity was meanwhile gaining ground in clinical medicine, largely because of investigations on hemolytic anemia (Dameshek and Schwartz 1938 and 1940) circulating anticoagulants ("Hemmkörperhämophilie —Deutsch 1950) and on Hashimoto s thyroiditis (Rose and Witebsky 1956, Roitt *et al* 1956). Theories on the nature of the immune system and on its failures (Burnet 1949 and later) and elucidating experimental work (Medawar Billingham *et al* 1953 and later) stimulated the new views intensely Regarding SLE, the primary cause was no longer sought in the connective tissue but in the immunologic apparatus. It was suggested that the term diffuse collagen disease be replaced by "complex autoimmune disorder (Dameshek 1958) or "systemic immunopathy" (Mackay and Burnet 1963). This invited comparisons with other diseases in which immunologic reactions might play a rol namely not only the other classic" collagen diseases, but also conditions such s Hashimoto s

thyroiditis, chronic hepatitis, and Sjögren's syndrome.

The chronic, self perpetuating nature of SLE and knowledge of the profound immunologic disturbances suggested that SLE might be an inherited constitutional abnormality. This incited a search for genetic factors, and in recent years familial investigations have been started to elucidate this aspect.

CLINICAL FEATURES

INCIDENCE AND PREVALENCE, RACE, SEX AND AGE

The increased interest in SLE following the diagnostic and therapeutic advances of the last decades has led to a marked rise in the annual number of cases diagnosed. But whether as claimed by some authors (Svanborg and Sölvell 1957) the true incidence of the syndrome really has increased, is still debatable. The annual *incidence* of newly diagnosed cases in New York City since 1955 had according to Siegel *et al* (1961) remained roughly constant about 10 per million inhabitants, while the *prevalence* had increased successively from about 25 per million in 1955 to about 40 per million by 1958. In this epidemiologic study SLE was found to be more common in *non-whites* than in *whites*.

SLE shows a clear predilection for *females*. In most series on record females represent 80—90 per cent of the patients (Table 2).

SLE occurs in all *age* classes, but its onset is most common between the ages of 10 and 40 years (Table 2).

MANIFESTATIONS

In the tables given by Harvey *et al* (1954), Dubois (1953) and Larson (1961) on the *initial* manifestations, joint symptoms are the most common, skin rash occupying the second or third position in order of frequency. Other initial symptoms or signs mentioned in the literature are pleuritis, pericarditis, nephropathy and false-positive serologic reaction for syphilis (Haserick and Long 1952). As pointed out by Harvey *et al* (1954) for example the initial manifestations often occur months or even years before the episode during which the diagnosis of SLE is made.

Symptoms and clinical findings *in the course* of SLE have been described in detail by various authors such as Harvey *et al* (1954), Talbott and Ferrandis (1956) and Larson (1961). The incidences of certain manifestations in some recent large SLE series are given in Table 2.

The *intermittent* course of SLE, in which acute exacerbations may cease spontaneously or be controlled by corticosteroid treatment, is usually described as a characteristic. Affection of various parts of the body simultaneously or successively is also a distinguishing feature. Most patients have *fever* and other general symptoms.

Certain manifestations such as *skin lesions* of LE type, *joint affection, enlargement of reticulo-endothelial organs, polyserositis, nephropathy* and *leucopenia* were regularly mentioned in the early descriptions of SLE. In recent years attention has been directed to certain relatively less common, but diagnostically valuable manifestations, *e g hemolytic anemia* and *thrombocytopenic purpura* (Michael *et al* 1951, Conley 1952, Dameshek and Reeves 1956), the *nephrotic syndrome* (Brenner *et al* 1948, Muehrcke *et al* 1961) and *false positive serologic tests for syphilis* (Coburn and Moore 1943, Zellman 1952, Harvey *et al* 1954). The examination for *LE-cells* is now regarded as the most important in

Table 2. Incidence of manifestations of SLE in 5 literature series[1]

Manifestation	Jessar et al[2] (1953)			Dubois (1953)			Harvey et al (1954)			Armas-Cruz et al (1958)			Larsen (1961)		
[Female sex]	[323	273	[illegible]]	[62	55	[illegible]]	[138	108	[illegible]]	[	—	]	[200	191	[illegible]]
[Age at onset (yrs)]	[10	—	40]	[20	—	30]	[10	—	40]	[	—	]	[10	—	30]
Fever	216	214	[illegible]	62	60	[illegible]	105	90	[illegible]	108	100	[illegible]		—	
Skin rash, total	327	271	[illegible]	62	53	[illegible]	105	69	[illegible]	108	83	[illegible]	200	101	[illegible]
Cutaneous LE	41	19	[illegible]	62	43	[illegible]	105	11	[illegible]	108	61	[illegible]		—	
Purpura		—					105	10	[illegible]	108	13	[illegible]		—	
Loss of hair	11	3	[illegible]	62	33	[illegible]	105	3	[illegible]	108	51	[illegible]		—	
Arthropathy	302	230	[illegible]	62	54	[illegible]	105	95	[illegible]	108	90	[illegible]		—	
Lymphadenopathy	216	69	[illegible]	62	26	[illegible]	105	61	[illegible]	108	50	[illegible]		—	
Splenomegaly	168	29	[illegible]	62	5	[illegible]	105	16	[illegible]	108	21	[illegible]	200	30	[illegible]
Hepatomegaly	164	20	[illegible]	62	21	[illegible]	105	31	[illegible]	108	26	[illegible]	200	51	[illegible]
Pulmonary lesions	41	9	[illegible]		—		105	17	[illegible]	108	58	[illegible]		—	
Pleuritis		—		62	37	[illegible]	105	60	[illegible]	108	49	[illegible]		—	
Heart lesions, total	211	114	[illegible]		—		105	58	[illegible]	108	96	[illegible]		—	
Pericarditis	214	45	[illegible]	62	27	[illegible]	105	17	[illegible]	108	13	[illegible]		—	
Raynaud's phenomenon	169	13	[illegible]	62	16	[illegible]	105	11	[illegible]	108	17	[illegible]		—	
Abdominal crisis	164	20	[illegible]	62	23	[illegible]	105	11	[illegible]	108	23	[illegible]		—	
Retinal lesions	41	9	[illegible]	62	20	[illegible]	105	32	[illegible]	66	20	[illegible]	200	20	[illegible]
Nephropathy		—		62	35	[illegible]	105	68	[illegible]	108	83	[illegible]		—	
Anemia	323	258	[illegible]	62	48	[illegible]	105	82	[illegible]	108	65	[illegible]	200	110	[illegible]
Leucopenia	323	220	[illegible]	62	12	[illegible]	122	71	[illegible]	108	17	[illegible]	200	35	[illegible]
Thrombocytopenia	175	18	[illegible]	62	6	[illegible]	86	23	[illegible]		—		196	15	[illegible]
Hyperglobulinemia	167	70	[illegible]	54	15	[illegible]	105	61	[illegible]		—		171	128	[illegible]
LE-cell phenomenon		—		60	41	[illegible]	96	79	[illegible]	92	83	[illegible]		—	
STS, false positive	323	58	[illegible]	62	19	[illegible]	138	38	[illegible]	108	10	[illegible]	190	58	[illegible]

[1] First figure in each group of columns: total number of cases in which the manifestation was sought. Second figure: number of cases with manifestation. Italics: per cent incidence of manifestation.

[2] The complete series of Jessar et al consisted of 44 personal cases and 279 cases garnered from the literature between 1918 and 1953.

OTHER POSSIBLY AUTOIMMUNE DISEASES

Some disorders with presumably autoimmune mechanisms have been discussed in association with SLE such as glomerulonephritis, allergic angeitis, myasthenia gravis, ulcerative colitis, sarcoidosis and amyloidosis. The LE-cell phenomenon is occasionally positive in most of these conditions, which also sometimes occur in otherwise typical SLE. Readers interested in a further discussion of such possibly autoimmune disorders are referred to Mackay and Burnet (1963).

THE CHRONIC FALSE POSITIVE STS REACTOR STATE

In occasional cases, a false positive serologic test for syphilis has been found to be an initial or early manifestation of SLE (Haserick and Long 1952).

Extensive investigations of primary materials of chronic false-positive STS reactors during the last decade (Moore and Lutz 1955, Miller *et al* 1957 Catterall 1961 Shulman 1963) have conclusively shown the frequent presence or later development of (1) the full SLE syndrome (2) RA or other definable collagen or autoimmune diseases or (3) a varying range of SLE-like symptoms and signs not permitting a definite diagnosis.

HYDRALAZINE SYNDROME

Hydralazine therapy in hypertension sometimes produces clinical side-reactions mimicking the SLE syndrome The hydralazine syndrome has been discussed extensively by several authors (see *e g* Comens 1961). The characteristics of the syndrome may be summarized as follows (1) It occurs in about 10 % of all patients treated with hydralazine (2) It is more liable to occur when the dose is large and given for longer periods. (3) Unlike the SLE syndrome it shows no predilection for either sex. (4) All degrees of severity are seen from slight arthritis to that of fullblown SLE. (5) The syndrome usually disappears after withdrawal of hydralazine but may sometimes persist for long periods. Characteristic histologic changes of SLE have been seen in some cases at necropsy (6) The LE-cell phenomenon is very often demonstrable in the hydralazine syndrome and is sometimes the only sign of an untoward effect of hydralazine (7) A condition resembling the hydralazine syndrome in man has been produced experimentally in animals. But neither the clinical picture nor the histologic findings correspond exactly to those of the hydralazine syndrome or of SLE in man (Braverman and Lerner 1962)

The relevant importance of the hydralazine syndrome lies mainly in the fact that it resembles SLE and that it is produced by an exogenous factor The possibility that hydralazine sometimes unmasks latent SLE has been discussed (Shulman and Harvey 1960). It is not known whether hereditary factors play any role in the causation of the hydralazine syndrome

DIAGNOSTIC CRITERIA

As mentioned in the preceding section, the concept of SLE has changed from time to time and has tended to become broader since the discovery of the LE-cell phenomenon. While some authors still believe SLE to be a well-defined clinical entity an increasing number of experienced clinicians and investigators regard SLE as a syndrome closely related to such disorders as arthritis and chronic discoid LE (see discussion in Immunologic Aspects .., 1963).

The difficulty in defining SLE is reflected by the lack of generally accepted diagnostic criteria. Formerly the diagnosis was made on the basis of morbid-anatomic findings thought to be pathognomonic and, clinically mainly on the basis of

characteristic skin changes. But other criteria were also suggested, those fairly wi dely used being the ones put forward by Brenner *et al* (1918). (1) The erythematous lesion of the skin, frequently in but terfly distribution of the face (2) constitutional symptoms of pyrexia, weakness, cachexia and loss of weight, (3) negative blood cultures (4) arthralgia (5) nephritis, (6) suppression of blood-forming elements, including leucopenia, secondary anemia and thrombocytopenia, (7) lymphadenopathy (8) endocarditis (non-bacterial) (9) eff sions into pericardial, pleural and less commonly the peritoneal cavities, (10) predominant occurrence in females. A firm diagnosis was usually regarded as requiring satisfaction of 7 of these criteria (Jessar *et al* 1953).

After the discovery of the LE-cell phenomenon, the demonstration of LE-cells was included as an important diagnostic criterion. In an attempt by the British Empire Rheumatism Association Pan l on Rheumatic Diseases to define the syndrome (see Weir *et al* 1061), the criteria were divided into tw groups, "major" and "minor The *major* criteria were (1) Rash compatible with disseminated lupus. (2) Leucopenia below 5000 (3) Finding of LE-cells in 15-minute search of one slide. *Minor* criteria. (1) Arthralgia or effusi ns into joints. (2) Serositis. (3) Fever above 99°F (37.2° C) twice in 24 hours. (4) Retinal changes. (5) Differential Agglutination Test 1 16 or more (6) Albuminuria above 5 mg/100 ml.

A similar classification of the manifestations was used by Dameshek (1960) who employed the terms diagnostic major and minor criteria. Discoid lupus, positive LE-cell test marked splenic perivascular fibrosis d wire-loop lesions in the kidneys were considered diagnostic Majo manifestations included idiopathic thrombocytopenic purpura Coombs positive hemolytic anemia, arthritis and nephritis. Dameshek accepted cases f idiopathic thrombocytopenic purpura with LE-cell almost certai ly SLE. In analogy with the terms used in the ARA criteria for rheumatoid arthritis, he used the epithets "definite" probable" and "possible SLE, according to the number and type of manifestations.

Siegel *et al* (1961) in their investigation of the epidemiology of SLE used the terms "definite" and suspected The *definite* cases had 3 or more systemic manifestations usually observed at some time in SLE, plus confirmatory laboratory evidence in the form of either a repeatedly positive LE-cell test or positive pathologic findings, *viz* typical "wire-loop" glomerular lesions, onion-skin splenic lesions, or hematoxylin bodies.

Most authors did not describe the diagnostic criteria applied but simply stated that the cases ran "a clinical course typical of SLE" and generally showed the LE-cell phenomenon.

SPECIFICITY OF THE LE-CELL PHENOMENON

Routine examination for the LE-cell phenomenon soon revealed that the phenome non was demonstrable in the vast majority of patients with clinically typical SLE (Haserick 1951 Sukata and Conley 1951 Harvey *et al* 1954). The LE-cell phenomenon was rarely described in other diseases and when it was, it was often thought to be due t misinterpretation of artefacts or to an atypical form of SLE. Harvey *et al* (1954) presented a list of 50 diseases in which they had not been able t produce the LE-cell phenomenon. The 79 positive results obtained proved to have occurred in patients with a clinical disorder they thought t be compatible with the diagnosis f SLE.

Further perience however showed that th LE-cell phenomenon was n t quite pathognomonic of SLE. But practically all the conditions in which various a thors had f und the LE-cell tests to be positive belonged to th gro p of collagen and utoimmune disea ses (Mont *et al* 1961). It is noteworthy

that, after SLE, rheumatoid arthritis is the condition in which a positive LE-cell phenomenon appears to be most common. Of 900 cases with LE-cells (Hijmans *et al* 1958) 144 were found to have been diagnosed as RA and 42 as SLE. The other positive results were found in patients with *suspected* SLE or RA, or other collagen diseases.

On the other hand, it has become evident that the LE-cell phenomenon is not an *obligatory* diagnostic criterion of SLE, for even recent SLE series include a small percentage of cases which had been accepted as SLE, though the LE-cell test had not been performed or had proved negative. Repeated examination over a long period has sometimes failed to reveal LE-cells even in cases in which the diagnosis was confirmed at later necropsy (Larson 1961 Rothfield *et al* 1961)

ETIOLOGY

Surveys of earlier concepts of the etiology of SLE have been given by Harvey *et al* (1954) Talbott and Ferrandis (1956) and Muehrcke *et al* (1961).

INFECTIONS

The name lupus erythematosus was probably coined because of a certain similarity of the appearance of the skin with that seen in skin tuberculosis, lupus vulgaris, a disease with which LE was formerly sometimes confused. The supposition of some relationship between LE and tuberculosis was apparently strengthened by the occasional finding of active tuberculosis of internal organs at necropsy of cases of LE. The concept of LE (and SLE) as a tuberculous disease persisted until far into the 20th century especially in Europe. Keil (1933) is generally credited with the elimination of this concept.

In recent years SLE and tuberculosis have again been discussed together because treatment of SLE with steroids is occasionally complicated by pulmonary and other types of tuberculosis and secondly tuberculostatics sometimes cause side effects resembling the SLE syndrome (Bickers *et al* 1961).

Of other *micro-organisms* mentioned in the discussion of the etiology of SLE are streptococci (Welsh 1918 Barber 1919) pleuropneumonia like organisms (Brown *et al* 1951) and viruses (Molten and Clark 1952). None of these micro-organisms, however has been proved entirely responsible for SLE. Various types of infections have been observed to precede acute bouts of SLE, and a causal relationship between *focal infection* and SLE has been postulated by Shearn and Pirofsky (1952) for example The infections have also been regarded as being of predisposing importance, possibly as a trigger mechanism of other pathogenetic processes (Harvey *et al* 1954)

ENDOCRINE FACTORS

Endocrine factors have been considered in the discussion of SLE for the following reasons (1) The syndrome shows a predilection for females and (2) it tends to make its first appearance in fertile age (3) pregnancy and menstruation have been observed to influence the clinical picture and the syndrome sometimes appears after parturition (Rose and Pillsbury 1944). Rose (1961) even suggested that the female mortality from SLE is correlated with the excretion of estrogen in the urine (4) The striking effect of corticosteroid treatment on SLE and (5) occurrence of a SLE like syndrome on withdrawal of steroids from patients with rheumatoid arthritis (Slocumb 1953).

In spite of the above-mentioned observations, no endocrine factors have proved entirely responsible for the development of

SLE, though they may have a predisposing effect (see Talbott and Ferrandis 1956)

HYPERSENSITIVITY TO EXOGENOUS AGENTS

The term hypersensitivity to exogenous agents was formerly used in the broad sense of the term as an excessive physiologic responsiveness to a (specific) chemical or physical agent. Now it is generally used to designate an *immunologic* process with consequent damage to the tissue (see discussion in Inflammation and Diseases of Connective Tissue, page 125 1961). The terms "allergy and "hypersensitivity" are often used synonymously Since the beginning of the 1930s SLE (Klinge 1933 and others) has been widely regarded as a hypersensitivity disease on th following grounds. (1) The general spread of the pathologic changes and their histologic appearance have been regarded as compatible with hypersensitivity (Klinge 1933, Rich 1946, Tellum 1918). Some pathologists, however assailed this opinion (Klemperer 1948, 1950). (2) SLE sometimes makes its appearance or is worsened by exposure to micro-organisms, and physical irritants such as sunshine and by treatment with vaccines, chemotherapeutics, antibiotics, and blood transfusions (Gold 1951). (3) Allergic reactions such as urticaria and drug exanthema are common in SLE. (4) SLE has been said to have become more common with the wider use of chemotherapeutics and antibiotics. (5) A clinical picture indistinguishable from SLE is sometimes produced by treatment with a well-defined chemical substance hydrala zine (see above)

The immunologic nature f the allergic reactions described has been supported by a number of observations made in recent years. According to Muelling *et al* (1958) and Harris and Vaughan (1960) patients with SLE have a marked tendency t form antibodies to antibiotics. In addition, som patients with SLE have proved to be able to produce antibodies against rare blood group substances (Callender and Race 1946) On immunization of a small group of SLE patients with Brucella antigen Meiselas *et al* (1961) found that the antibody response tended to be excessive —some of the patients also showed "non-specific" responses in the form of positive direct antiglobulin tests and appearance of thyroglobulin-antibody Other workers, however have had contradictory results. Muschel (1961) thus found no increase of normal isohemagglutinine titres, Proteus X 2 agglutinin titres or ASL-0 titres in 11 patients with SLE.

AUTOIMMUNITY

The discovery of the LE-cell phenomenon prompted intense research on immunologic phenomena in SLE. The LE-cell phenomenon was found to be produced by a gammaglobulin (Haserick 1950) with affinity for nucleoprotein (Miescher and Fauconnet 1954, Hijmans and Schult 1958 and 1959 Holman *et al* 1958 Klein *et al* 1959). Later a wide variety of serum protein factors were demonstrated with an affinity for other nuclear and cytoplasmic components (see surveys by Holman 1960 and Shulman 1963). Antibodies to erythrocytes (Michael *et al* 1951) leucocytes (Van Loghem *et al* 1958, Dausset *et al* 1961) and thrombocytes (Dausset *et al* 1961) were found to be frequent in SLE even in the absence of hemocytopenia. Antibodies to blood coagulation factors were occasionally found (Conley and Hartmann 1952, Nilsson and Wenckert 19.3). The false-positive serologic tests for syphilis (Coburn and Moore 1943) were ascribed to antibodies t phospholipids.

Common to these serum factors is that they react with normal constituents of the body and that they can be demonstrated by conventional serologic techniques including *e g* complement fixation. Further most f the serum factors involved have been shown t belong t the immunoglobulins (most being 7S some 19S gammaglobulins) Serum factors of this kind are

now conventionally called *autoantibodies* irrespective of their possibly pathogenetic importance (see Anderson 1963)

The concept of autoimmunity has been fertilized by theories on the mechanisms of immunology Such theories have been put forward by Ehrlich, Alexander Pauling, Burnet Jerne and Szilard (see surveys by Lederberg 1959 and Mackay and Burnet 1963). The theories produced by Burnet and associates have attracted most attention. According to Burnet s clonal selection theory" (Burnet 19 9), immunity results from the exogenous or endogenous antigen selecting a corresponding "clone of immunologically competent cells of the lympho-reticular system that then proliferates and produces antibodies. This theory assumes that every possible antigen determinant is already represented in the body by a clone of cells capable of producing corresponding antibodies The immunologic system can, however distinguish between exogenous (foreign, not self") antigens and own body constituents ("self" antigens) It is now generally accepted that this so-called immunologic tolerance" is acquired during fetal life and this statement has been amply substantiated by experimental work on transplantation immunity by Billingham Medawar and others (see Medawar 19o8). Burnet expressed the view that all immunologically competent cells capable of reacting with self" antigens, so-called forbidden clones undergo destruction during embryonic life But even during adult age forbidden clones may arise as the result of somatic mutation A homeostatic mechanism of unknown nature is, how er believed t destroy these forbidden clones before they have time to proliferate and produce n damage

Aut mmune disease is defined by Burnet as a condition in which structural or functional damag is produced by the action of immunologicall competent cells or antibodies against normal components of the body Two main types of aut immun disease ha been distinguished One type which Hijmans *et al* (1961) called "disturbed antigen" type is said to be due to certain body constituents with antigen properties, which, owing to anatomic conditions, are inaccessible to the lympho-reticular system and therefore fail to establish immunologic tolerance in early life A leakage of these antigens after trauma for example later in life results in proliferation of corresponding immunologically competent cells, and then the tissue is damaged either by the immunologically competent cells themselves (delayed type of hypersensitivity") or by the mediation of antibodies produced (antigen antibody reactions) The classic example on this form of autoimmune disease is Hashimoto s thyroiditis. As a rule this type of autoimmune conditions can be reproduced, under certain circumstances, in experimental animals.

The other type of autoimmune disease is ascribed to disturbed tolerance (Hijmans *et al* 1961), in which a disturbance of the homeostatic mechanism is believed to allow proliferation of forbidden clones against normally accessible antigens. As mentioned above in SLE a large variety of globulins capable of reacting with normal body constituents have been demonstrated. Most of these potential antigens are believed to be accessible to the lympho-reticular system and therefore normally tolerated. SLE are thus ranks as the systemic immunopathy" *par excellence* (Mackay and Burnet 1963). Human disease of this type is difficult, if not impossible to reproduce in animals

Hashimoto s thyroiditis and SLE are regarded by Hijmans *et al* as extreme examples at opposite ends of a spectrum of diseases showing varying immunologic disturbances. Other diseases such as rheumatoid arthritis, Sjögren s syndrome and chronic hepatitis show features of both types.

While the occurrence of serologic factors with antibody properties in SLE is established, their pathogenetic significance is still debatable It is, however generally

accepted that substances with affinity for blood cells can cause diseases of the blood. Circulating anticoagulants have been known to produce abnormal bleeding tendencies (see Margolius *et al* 1961). Antibodies to vascular endothelium might explain the widespread vascular changes in SLE, any ischemic damage then being secondary to such changes (Dameshek 1958), but the occurrence of antibodies to vascular endothelium cannot be regarded as proved. Antibodies against the glomerular basal membrane have been suggested as a cause of the common pathologic anatomic changes found in the glomeruli in cases of SLE. Other authors assume that the renal lesions are due to circulating antigen-antibody-complexes having been passively entrapped in the glomeruli (Dixon 1963).

Most interest has been focused on the antinuclear factors, possibly representing antibodies to something so fundamental as the constituents of the cell nuclei. These fact rs have been suggested to be responsible for the widespread tissue damage in SLE. Such antibodies do not, how ever appear to have any cytotoxic effect *in vivo* (see discussion by Holman 1959). Moreover in a few cases of clinically ac tive SLE it has not been possibl to demonstrate the LE-cell phenomenon or other antinuclear factors, and in patients with agammaglobulinemia a SLE-like syndrome has occasionally been observed (Good *et al* 1962). Antinuclear factors have been f und to pass through the placenta without any demonstrable injury to the fetus (Bridge and Foley 1954 Berlyne *et al* 1957) Arguments *for* the pathogenetic effect of antinuclear factors are as follows. These factors are demonstrable in *most* patients with SLE and are usually found in the highest titre during the acute stage f the disease (Mandema *et al* 1961 Townes *et al* 1963). Further pathologists have ascribed many of the histologic changes seen in SLE to antigen-antibody reactions (see page 12). The observation that the gammaglob lin is deposited in the glomeruli in LE nephritis (demonstrated by fluorescence technique by Mellors *et al* 1957) and that complement appears to be bound in the glomeruli (Lachmann *et al* 1962) is noteworthy. The complement level in the serum is often markedly low in SLE, particularly in the acute stages (Vaughan *et al* 1951) and this may be interpreted *inter alia* as a manifestation of antigen-antibody processes going on in the organism.

Another immunologic type of reaction may play a role in SLE and according to certain authors, a possibly more essential role than immune reactions mediated by circulating antibodies, namely the so-called cell-based delayed hypersensitivity reactions. This would be in analogy with the immunologic reactions to transplants —homograph rejection thus apparently being caused by immunologically competent cells rather than by circulating antibodies (see Medawar 1958). An observation regarded as arguing for such a mechanism in SLE is that a large proportion of patients with SLE show a delayed type of response to intra-cutaneous administration of autologous leucocyte suspensions (Friedmann *et al* 1960 Bennett and Holley 1961) According to Mackay and Burnet (1963), the occurrence of lymphocyte and plasma cell infiltrates in the tissues involved also support this assumption.

GENETIC FACTORS

The familial occurrence of chronic discoid LE has been known ever since the end of the 19th century (Hebra 1875) On perusal of the literature Leonhardt (1957) found that about 40 cases had been descri bed between 1901—1955. Some of them might in reality have been instances of SLE (Table 3) though in most cases data n systemic involvement are too meagre to warrant any definit conclusions. The first to report clinically clear-cut SLE in relatives were Davis nd Gutridge (1951), who described the occurrence of SLE in supposedly m nozygotic twins. This report and later publications with detailed case

Table 3. Reports of familial CDLE, with findings suggestive of SLE

Author	Year	Sex	Age at diagnosis	Relationship	Evidence of SLE
Legobbe	1937	F	33	Third	Fatal course Autopsy: polyserositis
		F	21	cousins	F tal course Autopsy: polyserositis
Grupper and	1955	F	58	Mother	Bouts f fever Persistent leucopenia
David		M	30	Son	Chronic false-positive STS. Leucopenia
Beckett and	19 9	F	61	Mother	Findings consistent with lupoid hepatitis
Lewis		F	32	Daughter	Hyperglobulinemia, ?nephritis
Steagall	1962	F	25	?Monozygotic twins	Arthritis, anemia. Previous purpura
et al		F	25		Previous purpura

extracts are listed in Tables 4—5 Several other cases have been casually mentioned by other authors (see Brunjes *et al* 1961).

In 19 7 Leonhardt published 3 cases of SLE in a sibship consisting of 14 members. In most of the other 11 siblings the serum gammaglobulin level was elevated, in 1 even excessively without subjective im pairment. An inherited tendency to over production of gammaglobulin with consequent SLE was postulated. The assumption of a genetically determined overproduction of (polyclonal) gammaglobulin was strengthened by examination of further members of this family which revealed differences between relatives of varying degree of relationship (Larsson and Leonhardt 19.9)

In the description of another instance of SLE in siblings, Larsson and Leonhardt (19.9) stressed the occurrence of joint symptoms in the patients with SLE and in some of their relatives, and a relationship between SLE and RA was supposed. The relatives were studied for rheumatoid factors, which were however not found to be substantially commoner than in controls consisting of their married part ners. Holman and Deicher (1960) also reported preliminary on an apparently increased occurrence of rheumatoid factors in relatives of patients with SLE.

Pollak *et al* (1960) found a definitely in creased incidence of antinuclear factors in relatives of patients with SLE about 50 % of 50 relatives studied by a fluorescence method showed positive titres.

Rodnan *et al* (1960) mentioned that they had found a few cases of false-positive serologic reactions for syphilis in SLE relatives. Such findings were also reported later by Morteo *et al* (1961)

Preliminary results of investigations of families with SLE have also been published by Siegel *et al* (1961) and by Ansell and Lawrence (1963).

The results of the investigations referred to above will be commented upon together with the findings made in the present in vestigation and in the last chapter the cumulative evidence of genetic factors in the etiology of SLE will be discussed.

Table 5. Reports of CDLE and SLE in newborn infants

Author	Year	Sex of infant	Clinical picture in infant	Clinical picture in mother
Mc Cuistion and Schoch	1954	M	CDLE (positive biopsy), transient	SLE (with positive LE cell test) developing 11 months after delivery
Hogg	1957	M	Died 2nd day after delivery: autopsy findings suggestive of SLE	CDLE, arthritis, hyperglobulinemia, negative LE-cell test
Dias et al	1958	F	Cutaneous LE and LE cells; fatal course	SLE (with cutaneous LE and positive LE-cell test)
Seip	1960		Hemocytolytic disease: apparent recovery at 2½ months	CDLE and severe hemocytolytic disease
Nice	1962	F	Hemocytolytic disease: positive LE-cell test, fatal course	Apparently normal
Meyer zum Büschenfelde	1963	M	"Full picture of SLE": positive LE-cell test	Apparently normal

Table 1 Report of familial SLE

Author	Year	Sex	Age at onset	Age at diagnosis	Relationship	Cutaneous LE	Positive LE-cell test	Positive autopsy	Classification of SLE	Remark
D I and	1951	F	18	19	?Monozygo-	Yes	NP*	NI	Definite	Zygosity
C trkige		F	?18	20	tic twins	Yes	Yes	Yes	Definite	not verified
Tikhonov	1951	M	15	18	Brother	Yes	NI	Yes	Definite	Miliary tbc SLE
Bugrov		F	17	18	Sister	Yes	NI	Yes	Definite	
Pirofsky	1953	F	11	30	Mother	Yes	NI	Yes	Definite	Miliary tbc SLE
and Shearn		M	17	17	Son	Yes	Yes	Yes	Definite	
Adams	1954	F	39	41	Mother	No	NP	?Yes	Suspected	Diagnosis in retro-
		F	27	30	Daughter	Yes	Yes		Definite	spect
Glagov and	1956	F	?20	11	Mother	Yes	Yes	NI	Definite	
Gechman		F	?	18	Daughter	Yes	Yes	Yes	Definite	
Agranat	1957	F	?27	32	Sister	No	Yes		Suspected	
et al		F	39	45	Sister	N	Yes		Suspected	
Leonhardt	1957	F	?13	17	Sister	Yes	Yes	Yes	Definite	Familial hyper
		F	21	36	Dizygotic	Yes	Yes	Yes	Definite	gammaglobulinemia
		F	?17	33	twins	Yes	N		Suspected	
Griffin	1958	M	13	16	Father	Yes	Yes		Definite	Son died from SLE?
et al		F	25	25	Daughter	Yes	Yes		Definite	
Lovada	1958	F	?18	10	Mother	Yes	Yes		Definite	
		F	? 8	10	Daughter	Yes	Yes		Definite	
Wagenhals	1958	F	21	22	?Monozygo-	Yes	Yes	NI	Definite	Zygosity
and Burgeson		F	26	26	tic twins	N	Yes	NI	Definite	not verified
Larsson and	1959	F	?29	31	Sister	Yes	Yes	Yes	Definite	Familial hyper
Leonhardt		F	?26	31	Sister	N	Yes		Definite	gammaglobulinemia
Glussani	1960	F	21	21	Sister	N	Yes	NI	Definite	
et al		M	18	18	Brother	Yes	Yes		Definite	
Marlow	1960	F	16	16	Sister	Yes	NP	Yes	Definite	
et al		F	15	15	Sister	Yes	Yes		Definite	
		F	50	62	Mother	No	Yes		Definite	

et al

et al		F	14	14	Daughter	Yes	Yes		Definite	
		M	38	38	Brother	No	Yes	NP	Definite	
		M	18	18	Brother	N	Yes		Suspected	
		F	28	28	Sister	N	Yes		Suspected	Familial hyper
		F	14	14	Sister	Yes	Yes		Definit	gammaglobulinem
		F	10	10	Sister	Yes	Yes	?Yes	Definit	
		F	11	11	Sister	Yes	Yes		Suspected	
		F	14	14	Sister	Yes	Yes	?Yes	Definite	
		F	?19	22	Sister	No	Yes		Suspected	
Blumenfeld	1963	F	15	16	Monozygo-	Yes	?Yes		Definite	
et al		F	?18	19	tic twins	N	Yes		Definit	
Lallet	1963	F	28	33	First	No	Yes	NP	Definit	
et al		F	19	28	cousins	?Yes	Yes		Definit	

By the present author NP = not performed

MATERIALS AND METHODS

SELECTION OF PROBANDS

The probands consisted of patients seen at hospitals in Scania 1955—1961 ("period of selection") with definite or suspected SLE according to the definitions given below.

1955 was chosen as the first year because examination for the LE-cell phenomenon had by then become generally accepted as a diagnostic adjunct in SLE, and laboratory resources for the examination had become available.

The population of Scania, the southernmost district of Sweden, was 875,839 by the end of 1958, the median year of the period of selection (*Statistisk Årsbok* 1960). Of these 473,315 (54.1 %) were living in towns, while 401,524 (45.9 %) were living in rural districts. Malmö, the largest town of Scania, had 221,000 inhabitants by the end of 1958. The population in Scania may be regarded as ethnologically uniform (Scandinavians). The number and distribution of physicians as well as the number of hospital beds available are satisfactory in all parts of the district. Practically all patients requiring hospital care receive it at hospitals *within* this district.

In the search for cases of SLE in the population during the above mentioned period the following sources were studied.

(1) *Hospital registers.* Owing to the variable clinical picture of SLE, patients with this syndrome may be admitted and registered under a variety of diagnoses. A search for the disease under all these possible names and in all hospital departments was regarded as impractical. It was therefore decided to search the registers for cases diagnosed as "systemic lupus erythematosus" or "collagen disease" at the departments where patients with SLE would most likely have been admitted, namely all departments of internal medicine, of pediatrics, of infectious diseases, of dermatology and of rheumatology in hospitals in Scania.

(2) *Biopsy and necropsy registers of departments of pathology.* The hospitals in the district under discussion generally send all biopsy and necropsy specimens to the Department of Pathology, Malmö General Hospital or the University Hospital, Lund, for histologic examination. These registers were sought for cases with the above-mentioned diagnoses.

(3) *LE-cell registers of hospital laboratories.* The registers of the results of examinations for LE-cells in the hospital laboratories were studied for patients in whom one or more positive LE-cell tests had been found.

From the above-mentioned sources all together 328 cases were found in which SLE had been suspected. All of the hospital records necessary for evaluation of the diagnosis were perused. Many of the patients with a provisional diagnosis of SLE in this primary material were eventually found to suffer from other diseases (such as infectious diseases, sarcoidosis, and cancer) and were excluded. Other patients were excluded because of inadequate clinical and laboratory information. Four cases of hydralazine syndrome were excluded because they presumedly represent a different etiology. This left

Tabl 6. Survey of primary and selected material

Category of patients	Males	Females	Total
Patients from Scania 1955—1961 with a definite or provisional diagnosis of SLE	66	262	328
Patients with a positive LE-cell phenomenon	12	87	99
Included in the proband series	9	63	72
Excluded	3	24	27
Rheumatoid arthritis with few systemic manifestations	*1*	*10*	*11*
Sarcoidosis	*1*	*5*	*6*
Hydralazine syndrome	*0*	*3*	*3*
Other drug allergies	*0*	*2*	*2*
Polyarteritis nodosa	*1*	*0*	*1*
Undiagnosed vague illness	*0*	*2*	*2*
SLE but sister already included	*0*	*2*	*2*
Patients with negative LE-cell phenomenon, or without LE-cell test	54	175	229
Included in the proband series	1	37	38
Excluded	53	138	191
Cutaneous LE with few systemic manifestations	*6*	*10*	*16*
Suspected SLE but sister already included	*0*	*1*	*1*
Other collagen diseases, other unrelated diseases, inadequate investigation	*47*	*127*	*174*

113 cases with a clinical picture consistent with SLE. Many of these cases had a "classical" clinical picture of SLE, whereas the diagnosis in other cases appeared less firm. In view of this and of the generally accepted criteria of SLE, it was decided to divide the material into 2 groups, namely probands with definite SLE, hereinafter called *group A probands* or *probands A* and probands with suspected SLE, hereinafter called *group B probands* or *proband B* The principles of this classification will be given below under the heading of "Study of proband
Three patients with SLE (1 definit and 2 suspected) were excluded from the proband series because each had a sist who had already been included The final material then consisted of 110 patients, of whom 57 were assigned to proband group A and 52 to proband group B One patient with definite SLE was reported separately as *proband KG.* Previous study of this patients' pedigree (Leonhardt 1957 Larsson and Leonhardt 1959) had disclosed an unusually high incidence of hypergammaglobulinemia and had prompted the present investigation. As this pedigree might possibly represent special circumstances it was thought better to treat it separately to avoid bias in the evaluation of the results in the new series.

The primary material, selected probands, and exclusions are summarized in Table 6.

In the evaluation f results given in later chapters the following remarks on the selection of the proband material should be borne in mind.

(1) The collection f probands does not includ *all* cases of SLE in Scania during th period in question (1953—1961) for the foll wing reaso s. It is probable that some individuals with SLE of benign

course did not seek medical advice or were treated at outpatient departments and therefore not noted in the hospital records studied. Some patients with SLE may have been treated at departments whose registers were not searched for SLE. The diagnosis of SLE must also be dependent upon the perspicacity of the examiners, their experience with the disease in question and the diagnostic criteria used. Many patients who might otherwise have been included as probands, were thus probably registered under diagnoses other than "systemic lupus erythematosus or "collagen disease" Incomplete investigation owing to limitation of laboratory resources and facilities for *post mortem* examination may also have resulted in some cases having remained concealed.

(2) The examinations for LE-cell phenomenon in the primary material had been performed at different laboratories and by different examiners. However the same method (Hargraves') had been used at the laboratories of the two university hospitals, as well as at most of the hospitals where the examination had been performed locally Random samples of positive preparations kept at the laboratories in question were checked by the author and found to be in good agreement with the generally accepted morphologic criteria (Hargraves 1954).

(3) Biopsy and necropsy specimens had likewise been examined by different pathologists. It was impractical to re-examine all preparations from the primary cases. As to those cases in which the general clinical picture was compatible with SLE, but in which the autopsy protocol did not contain unequivocal relevant diagnostic data the preparations were re-examined by the heads of the two departments of pathology in question (Prof C-G Ahlström of Lund and Prof F Linell of Malmö) In 5 cases the findings confirmed the diagnosis of LE. In 16 cases no diagnostic autopsy findings of SLE could be found, yet th medical history and the clinical findings were considered sufficiently characteristic to warrant acceptance of the cases as probands with "suspected" SLE.

As a further check of the *post mortem* diagnosis of SLE, those preparations that had been examined at the department of pathology in Malmö and in which typical findings had been described in the autopsy protocols were likewise re-examined (Prof F Linell). In no case did the re-examination indicate revision of the diagnosis.

The biopsy specimens consisted mainly of pieces of skin whose microscopic picture had sometimes confirmed the clinical diagnosis of cutaneous LE. A negative biopsy does not, however rule out such a diagnosis so that microscopic examination is of limited value (*Jessar et al* 1953) Re-examination was therefore considered unnecessary

(4) Alongside of the LE-cell phenomenon and the pathologic findings, cutaneous LE and then the "butterfly" exanthema in particular is the most important diagnostic finding in SLE. Evaluation of a face rash, however depends on the experience of the examiner In the primary material from which the probands were selected, face rash had been judged by different physicians, whose interpretations could, of course no longer be checked. In some cases, however the diagnosis had been confirmed by dermatologic consultants or by biopsy In all other cases the symptom was included as a weighty criterion for the diagnosis only if its appearance had been adequately described and especially if the rash had been reported as appearing after exposure to sunshine or during an exacerbation of the clinical course.

(5) Of other examinations, the results of quantitative determination of the serum protein fractions probably played an important role because in hypergammaglobulinemia there is reason to suspect SLE. F r serum protein analysis, facilities for paper electrophoresis were generally available during the period of selection (1955—1961). The examinations had, however been performed at different laboratories

with different apparatuses, so that the results are not strictly comparable. Judging from the normal values used at these laboratories, however the techniques used did not differ substantially from one another. Gammaglobulin values of 2.0 g per 100 ml or more were therefore taken as a measure of pronounced hypergammaglobulinemia and values of 1.5—1.9 g per 100 ml of slight hypergammaglobulinemia.

(6) At the Department of Internal Diseases in Malmö interest had long been focused on SLE and other autoimmune diseases, including overlapping syndromes. The material therefore includes a number of probands with features of both SLE and other diseases of suspected autoimmune nature such as rheumatoid arthritis, Sjögren's syndrome and chronic hepatitis. These cases were usually classified as suspected SLE (group B). More patients of this category would have been included in the present material if they had not been registered only under the last mentioned diagnoses, as they probably were at other hospitals.

(7) The departments searched for cases of SLE included the department for rheumatology in Lund, which receives mainly cases with pronounced symptoms of polyarthritis. The inclusion of this department might therefore have increased the number of probands with features of both rheumatoid arthritis and SLE.

STUDY OF PROBANDS

The symptoms and the laboratory results noted in the hospital records were recorded in tabular form (Appendix Table I), and the symptoms and signs were weighed according to their differential diagnostic value. Manifestations generally regarded as being typical of SLE were classified as diagnostic (D) certain pathologic changes (see below) positive LE cell phenomenon and cutaneous LE. The symbol ++ was used to designate symptoms and signs suggestive, though not typical of SLE. The symbol + was used for symptoms and signs generally accepted as compatible with SLE. Symptoms and signs that were little pronounced that had not been adequately described or that were possibly due to some other cause were designated (+).

The following symptoms and signs were regarded as manifestations of SLE *in the absence of other apparent causes.*

Remittent clinical course

++ Course markedly remittent
\+ Course remittent, but less markedly

Fever

++ Unexplained prolonged fever preferably with negative blood cultures, or several bouts of fever (morning temperature repeatedly >38 C)
\+ Few but marked, bouts of fever

Cutaneous LE

D Chronic discoid or acute-subacute LE, particularly with butterfly spread over face. If butterfly exanthema was not adequately described, the skin lesions were regarded as manifestations of cutaneous LE, only if dermatologic consultant had confirmed the diagnosis, or if biopsy had proved positive
(+) Hypersensitivity to sunshine, as judged from anamnestic data, without objectively recorded exanthema

Purpura

\+ Thrombocytopenic purpura; purpura hyperglobulinemica; other "vascular" purpura

Other exanthema

\+ Urticaria and different types of drug exanthema, particularly when recurrent

Loss of hair

\+ Pronounced loss of hair following an acute attack; loss of hair secondary to CDLE

Arthropathy

++ Arthritis of varying severity objectively registered, severe prolonged or recurrent arthralgia

+ Arthritis alone according to anamnestic data, moderate arthralgia

Lymphadenopathy

+ Significant generalized or regional enlargement of the lymph nodes

Splenomegaly

+ Spleen palpable and or roentgenologically enlarged

Hepatomegaly

+ Liver palpable and/or roentgenologically enlarged (in absence of cardiac incompensation)

Pulmonary lesions

+ Infiltration of pulmonary parenchyma of roentgenologically varying appearance—especially if recurrent—if not responding to antibiotic treatment but responding to steroid treatment

Pleuritis

++ Recurrent pleuritis, verified by positive physical signs and/or roentgenologic changes

+ Single attacks of pleuritis, verified as above—history very suggestive of pleuritis but without objective findings

Myocarditis

++ Clinically severe myocarditis with signs such as cardiac incompensation, gallop rhythm, roentgenologic enlargement of the heart, ECG changes. Recurrent, clinically mild myocarditis

+ Myocarditis producing ECG-abnormalities but only slight subjective symptoms

Pericarditis

++ Pericarditis, verified by positive physical findings and/or ECG-changes

Raynaud' phenomenon

+ Anamnestic data strongly suggesting Raynaud's phenomenon

Phlebitis, leg ulcers

+ Peripheral thrombophlebitis or thrombosis, particularly if recurrent, chronic leg ulcers (in the absence of gross varicose veins)

Abdominal crises

+ Recurrent attacks of abdominal pain (not caused by steroid-induced ulcers, gallstone etc)

Hepatitis

+ Chronic hepatitis or liver cirrhosis in association with other symptoms suggesting SLE

Sialadenitis

+ Objectively registered or history strongly suggestive of sialadenitis, particularly if recurrent

Keratoconjunctivitis sicca

+ Keratoconjunctivitis sicca diagnosed by ophthalmologist, with the aid of Rose Bengal staining or with Schirmer' test

Iritis retinopathy

+ Iritis, particularly if recurrent, cytoid bodies haemorrhages or exudates in the ocular fundi (in absence of systemic hypertension and/or uremia)—registered by ophthalmologist

Nervous system lesions

+ Attacks of psychosis; epileptic attacks (in absence of signs of uremia); organic lesions such as paraplegia

Nephropathy

++ Nephrotic syndrome with or without hypercholesterolemia; chronic nephritis with persistent proteinuria and/or abnormal urinary sediment, with or without increased NPN

+ Transient attacks of nephritis with signs as above particularly if recurrent

Anemia

+ Anemia (not caused by uremia or haemorrhage) with Hb below 11 g per 100 ml for men and below 10 g per 100 ml for women

Positive Coombs' test

+ Antiglobulin (Coombs') direct test positive

Leukopenia

++ WBC less than 3000 per cmm

+ WBC less than 4000 per cmm

Thrombocytopenia

++ Thrombocyte count less than 100,000 per cmm

+ Thrombocyte count less than 150,000 per cmm

Hemocytopenia was said to be present only when several values distinctly tended to be low and were below the respective limits on at least two consecutive occasions

Hypergammaglobulinemia

++ A serum gammaglobulin of the polyclonal type of 2.0 g per 100 ml or more on at least one occasion at investigation with electrophoresis on paper

+ A serum gammaglobulin value between 1.5 and 2.0 g per 100 ml

LE-cell phenomenon

D Several distinct LE-cells in a single preparation or few (but typical) LE-cells in more than one preparation. Samples recorded as "suspectedly positive" or with findings of only rosette phenomenon and/or free bodies were ignored

STS, false-positive

++ Positive serologic test for syphilis (Wassermann, Meinicke, Kahn, Kline), one or more with negative TPI-test

+ As above, but without TPI-examination; without historic evidence for syphilis

(+) Anticomplementary activity observed on performance of STS

Morbid-anatomical changes

D Libman Sack's endocarditis; well-marked splenic perivascular fibrosis, "wire-loop" lesions or focal glomerular necrosis in the kidneys

CLASSIFICATION OF PROBANDS

All the cases with diagnostic findings at necropsy also had a history consistent with SLE and were regarded as *definite* SLE. Cases with characteristic skin changes (cutaneous LE) *and* positive LE cell phenomenon were also classified as definite except one patient (proband 59) with but few signs of systemic involvement who was assigned to group B. Patients with only *one* diagnostic criterion in the form of either cutaneous LE or positive LE-cell phenomenon were allocated to group A, provided that the patient's medical history was judged as typical. This was considered to be the case if the patient had a constellation of symptoms and signs generally described in the literature as common in, or characteristic of SLE, particularly if they were remittent and responded favourably to ACTH or corticosteroids. These cases had manifestations scoring 9 + or more.

Patients without diagnostic manifestations (D) were allotted to group B *suspected* SLE. Patients with *one* diagnostic criterion were referred to group B if the medical history was judged as not quite typical of SLE. Thus cases of rheumatoid arthritis, Sjögren's syndrome and "lupoid" hepatitis were assigned to group B if these manifestations dominated the clinical picture despite the presence of some other in, manifestations suggestive of SLE. Patients, in whom necropsy had revealed no "diagnostic" changes were allocated to group B irrespective of other "diagnostic" manifestations and of the degree of systemic involvement. *Proband KG* who was reported separately had the 3 "diagnostic" criteria and a typical course of SLE.

Although the classification of some cases as "definite" or "suspected" SLE was rather arbitrary group A was intended to represent a collection of cases that most clinicians would have judged as definitely being affected with SLE. Group B, on the other hand was a more heterogenous collection of patients with a less typical history of SLE and patients with SLE-like syndromes.

SELECTION OF RELATIVES

In the investigation of genetic factors in SLE first degree relatives, aged 10 years or more (parents, siblings and children) of the probands were selected, and in the pedigree KG also nephews and nieces of the probands. Relatives of group A probands are hereinafter referred to as *group A relatives* or *relatives A* and relatives of group B probands as *group B relatives* or *relatives B*. The relatives of proband KG (*relatives KG*) will be treated separately.

Information on the number of the probands relatives and their places of resi-

dence was obtained from the probands, from other relatives, from the hospital records, and from the parish registers.

The investigation of the relatives was carried out during the years 1960—1963 (*time of study*)

STUDY OF RELATIVES

Classified according to availability for personal examination the relatives fall into 3 groups: (1) Relatives who were alive at time of study and residing in Scania (= study area) These relatives were interviewed personally by the author Blood specimens were collected for laboratory studies (see below). (2) Relatives who were alive at time of study and residing outside Scania. These relatives were interviewed by questionnaires sent by post. No blood specimens were drawn from these relatives, with the exception of 2 members of pedigree KG (3) As to relatives who had died before the study their surviving spouses or relatives were questioned whether the former had had rheumatic diseases. Furthermore, attempts were made to trace the cause of death in the parish registers and sometimes in hospital records.

PERSONAL INTERVIEW

All cooperating relatives of probands living within Scania at time of study were interviewed personally by the author They were systematically questioned regarding earlier diseases and any spells they had spent in hospital as well as their present state of health. Special attention was given to symptoms suggestive of SLE (see Chapter 4).

At the interview notes were also made of details not requiring extensive physical examination, such as the occurrence of face rash, goitre joint changes (particularly of the joints of the hands) acrocyanosis, signs of cardiac incompensation, general condition and state of nutrition.

INTERVIEW BY QUESTIONNAIRE PER POST

Relatives living outside Scania were interviewed by questionnaires containing questions regarding earlier severe diseases, earlier or present rheumatic complaints, any spells spent in hospital, and present state of health.

ANALYSIS OF HOSPITAL RECORDS

In those cases in which the history and the patient's appearance suggested previous or present SLE or related disease for which the patient had on some previous occasion been in hospital, the hospital records were procured and studied. The diagnosis and findings of interest in the point of view of the mentioned diseases were noted.

CLINICAL EXAMINATION

Of patients with SLE a few relatives who were living in Scania and in whom electrophoresis had shown pronounced hypergammaglobulinemia, and who had never been hospitalized, were requested to present themselves for examination at the Department of Internal Diseases in Malmö The examination comprised careful investigation of the general condition including examination of the joints, routine blood and urine analysis, chest X ray and in some cases also examination of the eyes and of the skin by specialists.

DATA FROM OFFICIAL REGISTERS

In Sweden all persons are registered from birth at their local parish office These registers contain notes on date of birth date of marriage and divorce, data on children on removal to or from the parish, and date of death. Since 1748 the registers contain a note of the cause of death—nowadays that given in the death certificates. The registers are usually kept

for about 50 years at the local parish offices and afterwards at the district archives.

Data were obtained by letter from the parish registers and the district archives were studied for the following data. (1) Number of first degree relatives of the probands and their identification data (name, date of birth, date of death) Technically the easiest information to procure was that on the probands parents, then on the children, while it was usually difficult to get complete data on the sibs. The pur pose of these data was to check information acquired from the probands themselves and from their relatives. (2) Cause of death of relatives of probands. The purpose of this information was to check the oral reports from the probands and their relatives, in some cases this was the only way to ascertain the cause of death. (3) Any consanguinity between the parents of the probands. This was checked by procuring identification data about earlier generations. The investigation was limited to the previous three generations of the probands. This was sufficient to reveal any marriages between first cousins. (4) Any relationship between different pedigrees detectable by the degree of infor mation obtained about generations preceding the probands generation.

SELECTION OF CONTROLS

The married partners of the probands and of the probands relatives were used as controls.

The married partners of group A probands and relatives were called *group A controls* or *controls A* those of group B probands and relatives were called *group B probands* or *controls B* The spouses of the members of pedigree KG were included in group A controls. The number of spouses and their places of residence were traced in the same way as the corresponding data on the relatives of the probands.

STUDY OF CONTROLS

Controls living at time f study (1960—1963) and residing in Scania were examined in the same way as the probands' relatives *i e* by personal interview and laboratory examination of blood samples.

Married partners who were living at time of study but not residing in Scania were not xamined. No investigation was made of the cause of death or earlier diseases of married partners who had died.

As in th investigation of the relatives, the records were procured f controls who had been in hospital for diseases possibly related to SLE.

None of the controls had such excessively high gammaglobulin values as some of the probands relatives. Thorough clinical examination was theref re not considered necessary

The advantages and disadvantages of a control material such as the ne used in the present investigation are discussed below

(1) The controls are available at the beginning of the investigation and have been selected by the experimental persons (the probands and their relatives) them selves. This voids any bias of the investigator owing to personal knowledge f the controls.

It is hardly likely that the marriages between the probands and their relatives and the controls were to any extent influenced by familial occurrence f SLE or f serologic abnormalities in either partner

(2) The prospect f cooperation may be regarded as better than for many other types of control series.

(3) The probands relatives and the

controls may be examined simultaneously and under identical conditions, an advantage which irons out any bias due to seasonal differences in serologic factors blood sampling or treatment of sera obtained.

(4) A control series consisting of married partners must necessarily be numerically smaller than the experimental material, in that some persons are not married, and others are divorced or widowed. Furthermore the parents of the probands cannot of course, be used as controls either

In the present investigation the numerical deficiency of the control material was compensated in various ways. To begin with the surviving married partners of deceased probands and of proband s relatives were, as a rule, included as controls. There were 2 groups of probands relatives, namely relatives A and relatives B. These groups were studied separately In the selection of controls for relatives A, however the corresponding control groups A and B were pooled provided that they did not differ significantly from one another in the respect studied. The control series was then even larger than the experimental group This also applies to relatives B. Finally the relatives of the proband KG were analysed separately but their married partners were included in control group A, which was thereby increased. The differences in size between the experimental groups and the control groups compared in the analysis imply no difficulty provided appropriate tests of significance are used.

(5) A control series should be selected with due regard to a number of variables (the "co-variables") to which the controls ought to be comparable with the experimental persons. The following variables were thought to be of special importance in this study-

a) *Age* SLE shows a certain age predilection, and the physical abnormalities and serologic factors under investigation may vary with age. A control series consisting of married partners largely represents the same age classes as the experimental series. Moreover any differences between experimental persons and controls can be eliminated by appropriate statistic methods.

b) *Sex* Sex must be considered in the investigation of pathologic conditions such as SLE with a strong predilection for one of the sexes, serologic abnormalities may also vary with sex. Controls consisting of married partners differ in sex from that of the experimental persons. However strict pairing of experimental persons and controls is not necessary The experimental series can be divided into groups according to sex (and age) and compared with corresponding groups of controls.

c) *Race* Racial differences probably occur in SLE (Siegel *et al* 1961) and may also influence the occurrence of serologic abnormalities. Such differences were however no problem in the present investigation, since both experimental persons and controls were of the same race (Scandinavians)

d) The family studies performed in this investigation were aimed to shed light over the relative importance of genetic and environmental factors in the causation of the SLE syndrome. Therefore the effect of environment on the physical and laboratory abnormalities studied must receive special consideration. The environmental factors of importance are difficult to define but might include such variables as the type of residence (whether rural or urban) hygienic conditions, dietary habits *etc* which might in turn involve different risks of exposure to infections chemicals and other exogenous agents.

An attempt was made to form an idea of the environmental factors by recording the residences and the socio-economic status of the experimental persons and of the controls. Also blood grouping was used to evaluate whether the different respondents represented a random selection of individuals.

It will be clear from the analyses to

follow that the 2 groups of controls, A and B differed regarding the environmental variables Since the incidences of the physical and laboratory *abnormalities* under investigation did not differ significantly between the 2 groups of controls, it was, however concluded that environments had no decisive influence on their occurrence or non-occurrence. It was then felt justified to pool the 2 groups of controls in comparisons with the experimental series.

BLOOD SAMPLING PROCEDURE AND TREATMENT OF SERA

Blood samples were obtained from probands, probands relatives and controls living in Scania at time of study (1960—63) All samples were collected by the author with the exception of a few probands sera collected while the patients were in hospital. The samples were drawn in the morning in the fasting state and centrifuged 4—5 hours later at room temperature, after which most of the serum was pipetted off The serum samples were frozen in glass tubes with rubber stoppers and kept at —15° C until analysed The blood cells with a small portion of serum were kept in a refrigerator for blood group examination, which was done within 3 days of sampling

The blood samples were numbered by the author in association with sampling The origin of the blood samples was unknown to the laboratory assistants who performed the subsequent analyses. Before microscopic examination for ANFs, which was done by the author personally the sera were re-numbered in such way that the examiner did not know from which person a given serum had been obtained.

Blood samples were collected from the controls (married partners) at the same time as from their relatives, and th samples from controls and from relatives were examined at the same time. Any effect of the time n the results of examinations would therefore have influenced the results of the samples from the relatives and the controls to the same extent.

LABORATORY INVESTIGATIONS

The following laboratory investigations were carried out on the blood samples collected from probands, relatives of probands and controls.

(1) Blood grouping (all blood samples were grouped ccording to th ABO-system the probands blood samples also according to the Rh-system)

(2) Electrophoretic analysis of serum proteins

(3) Test for the presence f rheumat id factors (SSC FII AP and FII SC-tests)

(4) Fl orescence test for the presence f ntinuclear factors

(5) Serologic test f syphilis (Wassermann s, M inick s and Kline s test)

(6) Determination of antistreptolysin titres

The methods f performing the above laboratory investig ti n will be described later in th chapters dealing with the results.

Completeness of laboratory investigations Owing t shortness f som sera o to technical mishaps, some laboratory investigations were not perf rmed on all respondents. H wever the lack of completeness in these instances was thought to be insignificant In the tables f results in th subsequent chapters the tot l number f sera investigated will usually be given.

STATISTIC METHODS

Arithmetic mean, standard deviation and standard error of mean were calculated according to usual statistic methods (Fisher 1916).

Variables not distributed according to the normal law were divided into 2 or more classes and chi square analysis was applied in comparisons between different distributions (Bonnier and Tedin 1957) When only 2 distributions were compared and each distribution consisted of only 2 classes, Yates' correction for continuity was applied.

The method described by Quensel and Gustafsson (1954) was used to obtain a chi-square for evaluating the significance of departure, when the main groups compared were divided into subgroups (e g according to sex and age)

A difference was said to be significant significant and significant when the corresponding levels of probability were 0,05—0 01 0 01—0 001 and less than 0 001 respectively

CLINICAL ANALYSIS OF PROBANDS

INCIDENCE AND PREVALENCE

The annual incidence of newly diagnosed cases of SLE (definite and suspected, respectively) during the period of selection is given in Table 7 Cases diagnosed in Malmö are listed *per se*. It is evident that relatively more cases of SLE were diag nosed in the hospital of Malmö than in the other hospitals of Scania. The difference is most marked regarding cases with suspected SLE and reflects the special interest in collagen diseases prevailing in the medi cal clinic of Malmö

An estimation of the real incidence of SLE might be done with most confidence from the figures given for Malmö which has only one hospital for acute illness and equipped with excellent diagnostic facili ties. It follows from Table 7 that the annual incidence of newly diagnosed cases of *definite* SLE has varied little the aver age being 10 per million per year which corresponds to the figure given by Siegel *et al* (1961) for New York City (see page 10).

The *prevalence* of all known cases of definite SLE in Malmö rose from 29 per million in 1955 to 45 per million in 1958 and to 60 per million in 1961. The corresponding figures for Scania except Malmö were 9 23 and 30 respectively The prevalence of cases selected as suspected SLE was, for Malmö 67 per million in 1955 and 82 per million in 1961 for Scania except Malmö th corresponding figures were 6 and 19 respectively

Also the figures for the prevalence of *definite* SLE in Malmö correspond well to those reported by Siegel *et al* for New York City

Table 7 Annual incidence of newly diagnosed cases

Year	Definite SLE				Suspected SLE			
	Malmö		Scania[a]		Malmö		Scania[a]	
	Cases	Rate[b]	Cases	Rate[b]	Cases	Rate[b]	Cases	Rate[b]
1955	2	8.6	3	3.5	7	32.5	3	3.5
1956	2	8.4	9	10.4	2	8.4	2	2.3
1957	2	8.2	7	8.0		32.3	3	3.5
1958	2	8.0	3	3.4	1	8.0	2	2.3
1959	4	17.7	2	2.3	3	13.3	4	4.6
1960	1	4.4	2	2.3	1	4.4	4	4.5
1961	3	12.9	6	6.8	1	4.3	3	3.4
Total	16	10.3	32	5.2	22	14.2	21	3.4

[a] Except Malmö. [b] Per million

Table 8. Mean ages of probands

Age	Group	No. in group	Mean age	SD	SE
Age at onset	Probands A	57	36.0	16.40	2.17
	Probands B	52	39.0	17.36	2.41
Age at diagnosis	Probands A	57	43 1	15.50	2.06
	Probands B	52	46.5	17 45	2.42
Age at study	Probands A	37[1]	43.7	13.27	2.18
	Probands B	37[1]	40 1	14 63	2.41
Age at death	Probands A	20[2]	48.3	18.79	4.20
	Probands B	15[2]	52.2	22.88	5.91

[1] No. of probands living at time of study
[2] No. of probands dead at time of study

RACE, SEX AND AGE

All the probands were *Scandinavians.* With but one exception, namely proband 43, whose ancestors were Danes, all were from families who had been living in Sweden, mostly within Scania, for at least 3 generations.

Fifty-one (89 4 %) of the 57 group A probands and 48 (92.3 %) of the 52 group B probands were females. The *sex distribution* thus agrees with that found in other materials of SLE in the literature (Table 2, page 11).

Table 8 gives *the mean ages* of the probands at the time of onset of the syndrome, of diagnosis, of the present study and of death of those who had died before the present investigation.

The *age at onset* is very approximate because it is difficult to know which symptoms or signs were the initial manifestations and secondly the patients could often not date the onset more than roughly. Symptoms such as arthralgia and Raynaud's phenomenon and signs such as increased ESR were taken as early manifestations of SLE, if they were a prominent feature also in the continued course. *Age at diagnosis* was calculated from the time when the diagnosis of SLE had been established *de facto* or when it could have been made with confidence on the basis of available clinical data. As a rule, the patient's age when a positive LE-cell phenomenon was first demonstrated or when the patient first showed typical butterfly exanthema was taken as the age at diagnosis. In the probands with suspected SLE (group B) there were often no "diagnostic" manifestations; and then age at diagnosis was calculated from the time when the diagnosis of SLE was actually suspected or could reasonably have been suspected from the clinical data available. *Age at study* was calculated from the time of collection of blood for the laboratory studies in the present investigation.

Although the differences were not statistically significant, the various mean ages of the probands were somewhat higher in group B than in group A. This reflects the fact that in the probands with suspected SLE the disease usually ran a more chronic course than in the probands with definite SLE. Also relatively more elderly women were included in group B where the criteria for inclusion were less rigid than for group A.

The *mean age at onset* was 36.0 years for probands A and 39 0 years for probands B. The peak of age at onset is given in large SLE series as usually between 20

Table 9 Interval between age of probands at onset of symptoms or signs and at diagnosis of SLE

Number of years	Probands A	Probands B
Less than 1	12	9
1—4	17	10
5—9	13	18
10—14	8	9
15—19	3	2
20 or more	4	4
Total	57	52

and 30 years (Dubois 1953 Harvey *et al* 1954 Larson 1961). It is evident that the estimation of the age at onset is very dependent upon the investigator's interpretation of symptoms such as arthralgia—whether as manifestations of SLE or as independent symptoms. In this respect, the present writer might have had a wider concept of SLE—as a constitutional disturbance with many facettes—than the cited authors. Also the difference may be due to the fact that only few children were included in the present series only 2 patients (group B) were less than 15 years at diagnosis. It is probable that a number of cases of SLE in children were registered under other headings than those studied by the author at children's departments of hospitals in Scania.

The *mean age at diagnosis* has not been calculated by other authors. The distribution of ages at diagnosis given by Merrell and Shulman (1955) for 99 patients from the series of Harvey *et al* appears not to differ much from that in the present series.

When *age at onset* and *age at diagnosis* are compared, it is apparent that usually several years elapsed before SLE was recognized (Table 9). The "delay in diagnosis" may be due partly to insufficient investigation of the patients, but mainly to the natural history of the syndrome which often develops during years, one system being involved after the other. As an illustration, the following case history is given.

Proband 1 (group A), female born 1930. In youth often infections, pneumonia, recurring tonsillitis. *1947* transient arthritis of the right radiocarpal joint, ESR 37 mm/1 hour. *1948* again arthralgia without objective joint changes but ESR 70 mm/1 hour—since then never lower values. *1950* thrombophlebitis of the right leg. ESR 92—100 mm/1 hour Meinicke reaction positive. Later in 1950 purpura of the legs and slight polyarthritis were noted, as were varying cardiac murmurs, which led to a diagnosis of rheumatic fever The thymol turbidity reaction was positive. Proteinuria was now occasionally recorded as were scanty red cells in the urinary sediment. Wassermann's test showed anticomplementary activity A longstanding ulcer of the left ankle was noted, and in 1951 a similar ulcer of the right leg. Later in *1951* the condition was aggravated with fever bilateral pleuritis, signs of myocarditis on ECG abdominal pain, and signs of renal insufficiency with maximum NPN 176 mg per 100 ml. The spleen was palpable, and the RBC and WBC were low Penicillin therapy was followed by exanthema, another exanthema appeared during administration of sulfonamides, and blood transfusion was followed by fever and hemoglobinuria. Coombs test was positive, and the coagulation time was over 5 hours. Electrophoresis (made for the first time) showed broad heavy gammaglobulin fraction (no values). The patient improved successively during cortisone therapy

In *1953* butterfly exanthema of the face appeared after exposure to sun. The spleen was palpated, and the blood values were again low (RBC about 2 million per cmm, WBC about 2000, thrombocyte count [illegible],000). Splenectomy was therefore performed. Histologic examination *of the spleen showed no onion-skin lesions.* The blood values improved, but 1 month after operation the patient had a spell of right-sided pleuropneumonia.

During the following years the patient had slight arthralgia, recurring thrombophlebitis and thrombosis of both legs, chronic leg ulcers and

Table 10. Interval between age of probands at diagnosis of SLE and at time of study or of death

Number of years	Probands A		Probands B	
	Alive	Dead	Alive	Dead
Less than 1	4	11	0	5
1—4	18	8	18	11
5—9	12	0	17	0
10—14	3	1	0	1
Total	37	20	35	17

longstanding butterfly exanthema. The renal affection gradually dominated th picture, and a *nephrotic syndrome with hypercholesterolemia* developed. This was complicated by signs of pyelonephritis. LE-cell phenomenon was first searched in 1952 and found t be positive for the first tim in *1953*

In the present material the interval between onset of symptoms and diagnosis was on the average 6.5 years for probands A and 7.5 years for probands B In Larson s series of SLE (Larson 1961) there was a "delay in diagnosis" of over 6 years in 22% of 200 patients. Other authors have not given detailed accounts of the time between onset and diagnosis but have in other ways stressed the often insidious onset and the chronicity of the syndrome (Harvey *et al* 1954)

Also the *interval between diagnosis of SLE and age at time of study or of death* (Table 10) illustrates the chronic course in many cases. Among the dead probands, many had been hospitalized in a terminal stage, which explains the shorter periods of observation in these columns.

RESIDENCE AND SOCIO-ECONOMIC STATUS

The distribution of the probands according to *place of residence* is given in Table 11 The difference between the 2 groups of probands lies at the upper significance limit ($\chi^2 = 5.96$, DF = 2, P = 0 05) and is explained by the fact that relatively more patients with *suspected* SLE had been found in the files of Malmö General Hospital which with few exceptions admits only patients from the town of Malmö

The *socio-economic* status of the probands is given in Table 12. Of probands B 60 % belonged to socio-economic class III against 50 % of probands A. The difference of the distributions was however not statistically significant ($\chi^2 = 2.83$, DF = 2, P > 0.20).

MANIFESTATIONS

The manifestations during the course of the illness are given in the Appendix, Table I where each proband is tabulated separately Table 13 shows the *incidences*

Table 11 Residence of probands

Group[1]	Total in group	Place of residence		
		Malmö	Other towns	Rural districts
Probands A	57	20	13	24
%		35.1	22.8	42.1
Probands B	52	30	6	16
%		57 7	11 5	30.8

[1] Males and females pooled, all ages

Table 12. Socio-economic status of probands

Group[1]	Total in group	Socio-economic class		
		I	II	III
Probands A	57	4	25	28
%		7.0	43.9	49.1
Probands B	52	6	15	31
%		11.5	28.9	59.6

Males and females pooled; all ages

of some of the manifestations in the groups of probands A and B. Statistically significant differences were found regarding the following manifestations: cutaneous LE, other exanthema, loss of hair lymphadenopathy phlebitis-leg ulcers, keratoconjunctivitis sicca, nephropathy anemia, leucopenia, and LE-cell phenomenon

The differences are due to the principles for selection of probands. *Cutaneous LE* and a *positive LE-cell phenomenon* were regarded as diagnostic" criteria and therefore often resulted in classification of a case as definit SLE. The higher incidences f *other exanthema loss of hair nephropathy anemia*, and *leucopenia* in probands A reflect the usually more serious course in the former This is also discernible in the manifestations *remittent course* and *fever* It is true that the frequ ncies here were the same for both groups of probands, but 31 (54 4 %) of group A probands had *pronouncedly* intermittent (++) course against 15 (28.8 %) of group B probands, a significant difference ($\chi^2 = 6.17$ DF = 1 $P < 0.05$). Thirty (52.6 %) probands A had prolonged (++) f ver against 16 (30 8 %) probands B ($\chi^2 = 4.39$ DF = 1 $P < 0.05$). On examination of the manifestation anemia Coombs test was positive in 10 of group A probands, against only 1 in group B The leucopenia also tended to be more severe in group A than in group B probands (40 1 % ++ leucopenia in group A against 25.0 % in group B). The higher incidences of *lymphadenopathy* and *phlebitis and leg ulcers* i probands A might also indicate a higher degree of severity of the illness or might simply be due to the fact that probands A had also been selected because of the *multitude* of their symptoms and signs. Most of the other manifestations were numerically more common in group A than in group B probands, although the differ ences were not statistically significant Group A probands had on the average a score of 15.2 + per proband, th corresponding figure for group B being 12.2 +

Group B probands showed a higher incidence of *keratoconjunctivitis icca* than group A probands, while cases of Sjögren s syndrome were intentionally incl ded in group B with suspected SLE. Three probands in group A, however also had this manifestation. For the same reason probands B had a higher percentage of sialadenitis than probands A, the difference was, however not statistically significant. Group B also included 6 cases of (chronic) *hepatiti* but liver cirrhosis was also found in 4 f the group A probands.

It is noteworthy that the frequency of *hypergammaglobulinemia* and *STS false-positive* was the same in both groups of probands.

In table 13, the range of per cent incidences f th different manifestations in the 5 literature series (Table 2) is given for comparison. It is clear from the table that the incidences of different ymptom and signs vary markedly i the literature series, which may be due to differences in criteria for selection f cases, to differences

Table 13. Incidences of manifestations in probands

Manifestation	Other series %	Probands A (57)		Probands B (52)		Difference between groups of probands	
		No.	%	No.	%	χ^2	P
Remittent course	—	52	91.2	44	84.6	0.65	>0.30
Fever	86—99	50	87 7	48	92 3	0.20	>0.50
Cutaneous LE	39—69	28	49 1	8	15.4	12.38	<0.001
Purpura	9—12	11	19.3	9	17.3	0 072	>0.70
Other exanthema	—	34	59 6	18	34.6	6 78	<0.05
Loss of hair	3—62	14	21 6	2	3.8	7 60	<0.01
Arthropathy	78—92	53	93.0	48	92.3	0 018	>0.80
Lymphadenopathy	32—58	26	45 6	13	25.0	4.10	<0 05
Splenomegaly	8—19	13	22 8	13	25.0	0,066	>0.70
Hepatomegaly	12—34	9	15.8	11	21.2	0.20	>0.50
Pulmonary lesions	20—54	34	59 6	23	44.2	1 96	>0 10
Pleuritis	45—60	40	70 2	33	63.5	0.27	>0.50
Myocarditis	—	16	28.1	8	15 4	1 81	>0 10
Pericarditis	6—45	12	21 1	10	19 2	0.056	>0.80
Raynaud-like symptoms	8—26	13	25 0	11	21.2	0 043	>0.80
Phlebitis, leg ulcers	—	16	28.1	5	9 6	4.73	<0.05
Abdominal crisis	10—37	7	12.3	5	9 6	0.20	>0.50
Hepatitis	—	4	7.0	6	11.5	0.21	>0.50
Sialadenitis	—	5	8.8	11	21.3	2.34	>0.10
Keratoconj sicca	—	3	5.3	14	26.9	7.98	<0.01
Iritis, retinitis	10—32	7	12.3	2	3.8	1 48	>0.20
CNS lesions	25—37	6	10.5	4	7 7	0.26	>0.50
Nephropathy	57—77	32	56.1	12	23.1	10,90	<0 001
Anemia	60—80	36	63.2	22	42.3	3 85	<0.05
Leucopenia	18—68	44	77.2	29	55.8	4.63	<0.05
Thrombocytopenia	8—27	18	31 6	9	17.3	2.19	>0.10
Hypergammaglobulinemia	—	51	89.5	47	90 4	0.025	>0.80
LE-cell phenomenon	69—96	43	75.4	28	53.8	4.59	>0.05
STS, false-positive	9—33	9	15.8	8	15.4	0.003	>0 95

See Table 2

in examination of the patients and to different conceptions of manifestations such as "pulmonary lesions" and "anemia". As to the incidence of *purpura* figures are given only in 2 of the literature series—9 % in Harvey's material and 12 % in Armas-Cruz' material. In the present proband group A the incidence was 19 % and thus did not differ markedly from those cited. The incidence of *arthropathy* in the present group A was roughly the same as those in 3 of the series on record—Dubois and Harvey 90 % each, Armas-Cruz 92 %—while only 1 series showed a lower incidence—Jessar 76 % (no data given for Larson's series). The incidence of *splenomegaly* was slightly higher than the highest given in the literature—19 % in Armas-Cruz' material as against 23 % in group A probands. In probands A the percentages of both

pulmonary lesions and pleuritis were somewhat higher than in any of the series on record, th differences were however not large. In the present material the inci dences of leucopenia and thrombocytopenia were higher than those in any of the published series. It was, however not possible to establish the *true* incidence of thrombocytopenia in the published series because in none had thrombocyte counts been made in all patients. As to the incidence of *leucopeni* there may be a real difference. The limit used in the present investigation, WBC 3000 per cmm, is lower than that used by other authors (Dubois, Larson 4500 and Jessar Harvey 4000). In Harvey's material of 122 patients 71 (58.2 %) had "counts less than 5000" but only 25 (20.5 %) counts less than 3000" No explanation can be offered fo this difference

As to the *CNS lesions* the incidence is much lower in the present proband group A than those given in the literature This may be due to the fact in this investigation epileptic forms of attacks were not considered as manifestations or SLE when the patient had co-existing uremia of final coma. In addition only gross psychotic states that occurred during acut exacerbations were incl ded.

The gro p B probands had slightly higher incidences of *purpura, splenomegaly* and *pleuritis* than the five published series. The incidences f *cutaneous LE lymphadenopathy CNS lesions nephropathy anemia* and *LE-cell phenomenon* were lower than in th published series. The differences seem to be true and due to the method of selection of probands B, whi h do not represent classic SLE but a heterogenous group f SLE-like syndromes, often with a lower degree of systemic involvement. With regard t the wide variati n of incidences f SLE manifestations i the literature series, it might be concluded that the group A probands did not differ essentially from those cited. The gro p B probands, on th other hand, had on the whole a lower degree of systemic involvement.

Some manifestations of SLE and other findings in the probands of differential diagnostic or etiologic interest are discussed below in the order they are given in the Appendix, Table I. Also some findings not given in Table I are included in this discussion.

CHRONIC DISCOID LUPUS ERYTHEMATOSUS

Twenty-eight (49 1 %) of group A and 8 (15.4 %) of group B probands were reported to have cutaneous LE. The diag nosis was confirmed by biopsy in 3 probands A and in 2 probands B, and dermatologic consultants had consented to the diagnosis in an additional 6 probands A and 3 probands B In about half the cases the cutaneous eruption was longstanding and was then often labelled as "chronic discoid"

In 4 probands A and in 2 probands B the skin lesion had been present for one year o longer before the diagnosis of SLE was established o suspected because of other manifestations (Table 14). The dif ficulties in the differential diagnosis between CDLE and SLE are further brought out by the following case reports:

Proband 10 (group B), female born 1901 Since about *1948* recurrent face rashes, usually on exposure to sunshine In *1953* CDLE was diagnosed by dermatologist. In *1958* arthralgia and loss of bodyweight. ESR only 20 mm/1 hour but gammaglobulin level 1.9 g per 100 ml. Subsequently the patient had slight arthralgia as well as nervous troubles. I *1959* gammaglobulin level as before, but LE-cell phenomenon now positive In *1961* still diffuse arthralgia without objective signs. Again positiv LE-cell test and hypergammaglobulinemia (1 7 g per 100 ml); ESR 56 mm/1 hour Ophthalmologist report. symptoms and signs typical of keratoconjunctivitis sicca.

Proband 11 (group A), female born 1896. In *1934* for the first time rash on both cheeks, but for several years occasionally rash of the scalp I *1944* the diagnosis of CDLE w esta blished clinically ESR, how er only 6 mm/1 hour Leucopenia with lowest value 2900 w

Table 14. Some manifestations in probands antedating diagnosis by years

Manifestation	Group	Total with manifestation	*Number having manifestation before diagnosis*		
			1—4 yrs	5—9 yrs	≥10 yrs
Cutaneous LE	Probands A	28	1	2	1
	Probands B	8	1	0	1
Joint involvement	Probands A	53	15	12	8
	Probands B	48	8	14	7
Increased ESR	Probands A	57	14	13	9
	Probands B	52	10	14	6
Hyperglobulinemia[1]	Probands A	51	16	6	0
	Probands B	47	9	2	0
STS, false-positive	Probands A	9	2	0	1
	Probands B	8	3	1	0

As estimated by electrophoresis on paper or by other methods of serum protein fractionation

noted. Exanthema improved on treatment with ACTH and cortisone but recurred on withdrawal of these drugs. In *1946* left-sided bronchopneumonia, ESR 46—63 mm/1 hour WBC 3300 and a faintly positive Meinicke's reaction. Later in 1956 poorly healing leg ulcers (no varices). The patient also complained of arthralgia. In *1951* in addition to butterfly exanthema, moderat swelling of the superficial lymph nodes, hypergamma globulinemia (1 6 g per 100 ml) and leucopenia (2400—4100) were recorded Since 1958 arterial hypertension was known, and in 1957 cardiac incompensation supervened, from which the patient died. *Post mortem* showed *inter ali* focal glomerulitis and "onion-skin lesions of the splenic arteriolae.—*No search had ever been made for* LE-cells.

One patient had a severe attack of SLE with cutaneous manifestations. She im proved but has later had cutaneous and other "chronic" manifestations possibly related to her basic abnormality·

Proband 40 (group A), female born 1909 had in *1948* a severe disease with cachexia, disorientation, polyarthritis, recurrent bouts of high fever bilateral pleurisy necessitating repeated puncture varying bilateral lung parenchymal changes, h pat megaly nd signs of nephropathy with proteinuria, microscopic hematuria and granule casts in the sediment. Red infiltrates around the the nails and red cyanotic patches in the face were interpreted by dermatologist as probable cutaneous LE. The ESR was increased t 110 mm/1 hour and anemia with Hb about 10 g per 100 ml, leucopenia with values down t 1900 and positive Takata's reaction were noted. The patient was treated mainly with sulfonamides and salicylate preparations and gradually improved At follow-up in *1953* she was in a fairly good general condition, b t stabbing pains in the chest persisted, the ESR was 38 mm/1 hour and there were still signs of nephropathy In *1955* the abnormal urinary findings had disappeared but the ESR was slightly levated (20 mm/1 hour).

In 1918 early cancer of the uterine cervix was diagnosed was treated radiologically and has since shown no *signs of recurrence In 1951* superficial thrombophlebitis of the right lower leg and the following years several such attacks of thrombophlebitis in both legs. I 1952 again erythema f the fingers and blue-red discoloration f the nose; th dermatologist diagnosis was LE.

The f llowing years the patient f lt well apart from few short bouts of fever stiffness f the fingers i th morning and Raynaud's phenomen on f moderate trength She also had new attacks of thrombophlebitis, and a slowly healing ulcer f the right foot appeared in *1959* Laboratory investigation showed ESR about 50 mm/1 hour gammaglobuli 1 6 g per 100 ml, moderat leucopenia (WBC of about 3000). There was, however no positive LE-cell phenomenon or evidence f nephropathy During further observa tion until 1900 the patient felt well b t the tendency t thrombophlebitis persisted as did the levation of the ESR (30—60 mm/1 hour)

The occurrence of a chronic discoid type of skin rash in a patient with otherwise "classic" SLE is illustrated by the case report of proband 9 (see page 55).

PURPURA

Eleven (19.3 %) of group A and 9 (17.3 %) of group B probands had skin lesions de scribed as purpura. Of these probands, 7 of group A and 2 of group B had concomitant thrombocytopenia. Three probands B (numbers 61 84 and 07) had longstanding purpura of the type described by Waldenström (page 14).

Proband 44 (group B), female born 1931 In *1944* an increase of the ESR t 100 mm/1 hour was noted in association with infection of the upper respiratory tract, and on later occasions the ESR was never found to be normal. In *1946* pain and swelling of the knee joints as well as peripheral arthralgia developed. This was ac companied by small bleedings together with a burning sensation in both legs. Hemorrhages recurred continually during the following years, often on physical exertion, exposure to sunshine and psychic stress.

In *1950* right-sided pleurisy and in *1953* and *1954* several attacks of abdominal pain and fever. On examination in 1954 ESR between 62 and 90 mm/1 hour and gammaglobulin 2.9 g per 100 ml, and 2 LE-cell tests positive. Still attacks of purpura, despite normal thrombocyte counts. In *1961* again joint pains: on examination symmetric peripheral polyarthritis. ESR 76 mm/1 hour gammaglobulin 2 6 g per 100 ml. WBC lowest 3200 A few attacks of fever were readily controlled by corticosteroids.

Recurrent purpura, probably of th same nature, was present in proband 14 (page 49), belonging to group A.

Purpura is described as a manifestation of SLE in the large literature series but is usually ascribed to thrombocytopenia or to allergy (Harvey *et al* 1954).

ARTHROPATHY

In both groups of probands *arthropathy* was equally common as remittent course and f ver The type of joint involvement in the probands was described in accord nce with Freedman *et al* (see Review) as (1) arthralgia and myalgia (2) acute or subacute polyarthritis and (3) chronic polyarthritis. The distribution of the various categories is given in Table 15. The dif ference between group A and B was significant ($\chi = 9.09$ DF = 2, $P < 0.05$) and the higher number of probands with chronic polyarthritis in group B was due to selection, for patients with chronic deforming polyarthritis were inclu ded in group A only if the diagnosis of SLE was well founded, whereas questionable cases were referred to group B. Only 3 (5.3 %) of group A and 4 (7 7 %) of group B probands had been described as having no joint symptoms whatsoever Nine (15.8 %) of group A and 12 (23.1 %) of group B had only had arthralgia or myalgia, without any demonstrable objec tive symptoms. Thirty-five (61 4 %) probands A and 18 (34.6 %) probands B had acute-subacute attacks of polyarthritis, usually involving both large joints (shoulder-elbow-hand-knee-foot) and small joints (fingers and toes). Signs of joint involvement in the form of pain on motion, limit ed motion, or joint swelling, tended to be most common in association with exacer bation of other symptoms with which they often also abated, particularly during steroid therapy Often, however slight to moderate arthralgia persisted between exacerbations. The objective signs were reported in most cases as relatively slight in relation to the marked subjective symptoms. Roentgen examination of the joints of the hands in 9 cases showed no changes (8 cases) or only slight periarticular decalcification (1 case). Despite fre quent attacks of polyarthritis th patients with this type of joint involvement had no j int deformities.—Subcutaneous nodules had been observed in 3 of the probands (all of group A).

T n (17.5 %) of group A and 18 (31.6 %) of group B probands had *chronic* poly arthritis with permanent joint swelling and often deformities. Of 22 probands with this type of joint affection and examined roentgenologically (8 proba ds of group

Table 15 Classification of joint affection in probands

Type of joint affection	Probands A		Probands B	
	No.	%	No.	%
Arthralgia	9	15.8	12	23 1
Acute-subacute polyarthritis	35	61 4	18	31 6
Chronic polyarthritis	10	17.5	18	31 6
No joint ymptoms	3	5.3	4	7 7
Total	57	100 0	52	100.0

A and 14 of group B) 16 (6 probands A and 10 probands B) were described as having changes typical of rheumatoid ar thritis (cartilage and bone destruction subluxations) while 6 (2 of group A and 4 of group B) only had non-specific changes (periarticular rarefaction) Nodules were found in 3 from each group of probands.

Of the 10 probands in group A with chronic polyarthritis 3 had the diagnosis of SLE confirmed post mortem 3 had both the LE-cell phenomenon and "diagnostic" LE eruptions, while 4 had LE cells and a degree of systemic involvement scoring from 14 to 20 + —Of the 18 probands B with chronic polyarthritis, 11 had positive LE-cell phenomenon, 4 cu taneous LE, they had a degree of systemic involvement scoring from 7 to 21 + Three probands B had no diagnostic manifestations but systemic involvement scoring 13, 16 and 19 + respectively

Symptoms and signs of joint involvement were thought to be the initial manifestation of SLE in 29 (50 9 %) of group A and in 32 (61.5 %) of group B probands and thus constituted the most common ini tial manifestation by far They were often reported to have been present for years before the diagnosis of SLE. 8 group A and 7 group B probands had had joint symptoms for 10 years or more (Table 14).

Th difficulties met in the differential diagnosis between SLE and rheumatoid arthritis are illustrated by the following case history·

Proband 19 (group A), female born 1910. Onset of joint pain *1932* bjective signs of polyarthritis *1933* when th ESR was only 3—7 mm/1 hour 1937 1940 and 1951 th patient received aurotherapy The ESR *markedly* levated, 68—128 mm/1 hour for the first time in *1945* Then also slight anemia (Hb 10.4 g per 100 ml), WBC 3000 and anticomplementary activity in Wasser mann reaction. During gold therapy (in 1915) bout of dermatitis.

Polyarthritis ran a chronic progressive course. In *1952* penicillin therapy of decubital ulcers was followed by prolonged fever that did not respond to antibiotics. The patient also had anemia, moderate leucopenia (lowest value 3200), increased ESR (68—78 mm/1 hour), positive SSC-test (640), and intermittant proteinuria. ACTH nly had temporary effect on joint pain and increased ESR. In *1954* commencing splenomegaly was noted, as well as anemia (Hb 7 4 g per 100 ml), leucopenia (WBC about 2000), hypergammaglobulinaemia (3.2 g per 100 ml) and typical LE-cells in peripheral blood smears. I *1956* infection of the respiratory tract was follow ed by a croup-like condition in which the pa tient died. *Necropsy* showed acut laryngitis, signs f previous bilateral pleurisy and renal changes with typical "wire-loop lesions.

In view of the fact that the diagnosis of "definite rheumatoid arthritis" according to the generally accepted criteria (Ropes *et al* 1959) is satisfied by the observation of peripheral symmetric joint swelling last ing for more than 6 weeks it is evident that all the probands with "chronic poly arthritis" and many of the probands with "acute-subacute polyarthritis would have been labelled with this diagnosis if they had not had other manifestations consist ent with SLE. In many cases (as illustrat ed by the case reports) the diagnostic la belling seemed arbitrary

Table 16. Distribution, among negative (—), borderline (±) and positive (+) results, of SSC-test in probands. Compilation of previous results noted in the hospital records

Group[1]	Total tested	Total No. of tests	SSC-test —	±	+
Probands A (57)	48	168	59	44	65
		%	35.1	26.2	38.7
Probands B (62)	45	177	54	38	85
		%	30.5	21.5	48.0

[1] Males and females pooled all ages

While the incidence of unspecified "arthropathy in the present probands was the same as that in large SLE compilations (see above), it is difficult to form an opinion of the proportions between *different types* of joint involvement in the different series. The proportion between cases with arthralgia only and cases with clinical signs of joint involvement is thus only vaguely given in the reports of Jessar *et al* (1953) Dubois (1953), Harvey *et al* (1954) and Armas-Cruz (1958). Of the 200 cases of SLE compiled by Larson (1961) 40 met the American Rheumatism Association criteria for the diagnosis of definite rheumatoid arthritis except that a positive LE-cell preparation was found in 38 of th m which corresponds to an incidence of 20 %. But nothing is said whether the other SLE patients had any objective signs of joint involvement. Shearn and Pirofsky (1952) found "varying degrees of heat, swelling and local redness" in more than half" of their 34 cases. Of Copeland s (1953) 47 cases 28 % were described as having arthralgia only" and 59 % arthritis

Cases with chronic, often deforming, joint affection are clearly included in the literature series of SLE, but the incidence appears to vary widely Jessar *et al* found a permanent clinical or X ray idence of joint involvement in 34 % of their personal 41 cases, Harvey *et al* typical rheumat id with deformity in 27 % f 105 patients and Armas-Cruz *et al* "joint deformities in 22 % of 108. Other authors state that progression to rheu matoid-like arthritis is unusual" (Mackay and Burnet 1963)

In the section dealing with the selection of probands it was pointed out (page 29) that the inclusion of a special clinic for rheumatic diseases among the hospital departments searched for primary cases might have increased the number of probands with chronic polyarthritis as an out standing feature of their illness. This type of joint involvement was present in 17.5 % of group A and 34.6 % of group B probands (see page 45), but these figures do not seem to be unusually high when compared with those in many published series.

A large number of the probands had been examined for *rheumatoid factors*. The SSC-test was the commonest method used, and most of the examinations had been performed at one and the same laboratory (Department of Clinical Bacteriology Malmö)—the remainder had been examined t the laboratory in Lund, where the same method is used and the same titre (64) is given as the lowest posi tive In several cases repeated determina tions had been performed in the course of the disease

Table 16 shows all results of SSC test reported in the hospit l records. The va l es have been distributed among 3 groups—negative (titre 0), borderline (titres 16—32) and positive (titre 64 or higher). It is seen that positive values were numeri-

cally more common in probands B There was, however no *significant* difference between the 2 groups of probands regarding the distributions of results of SSC-test (χ = 3 09 DF = 2 P > 0 20)

Positive tests for RFs have not been included as a manifestation of SLE in Table I Appendix.

CARDITIS

The constellation of acute-subacute polyarthritis and signs of heart affection (murmurs, arhythmia electrocardiographic and roentgenologic changes) in the probands had often led the treating physicians to consider *rheumatic fever* as a differential diagnosis. A few probands had recurring attacks simulating rheumatic fever only to later on develop other manifestations securing the diagnosis of SLE.

Proband 81 (group A), female born 1925. Since the age of 9 years often tonsillitis. An attack in *1947* was followed by fever transient arthritis, nodous erythema at the right elbow prolonged trio-ventricular conduction time on ECG systolic murmur on auscultation, and ESR 52—13 mm/1 hour The ASL titre was not increased, but hemolytic streptococci were isolated from the pharynx. Despite tonsillectomy in 1948 recurring sore throat and transient arthralgia. In 1949 bronchopneumonia, ASL 1250—250 U After pharyngitis in 1951 again arthralgia (principally elbows and knees), nodous erythema of the right arm and both legs, ASL up to 500 U faint systolic murmur but no ECG changes. During the following years, occasional spells of sore throat and arthralgia, treated with penicillin. During penicillin treatment in *1953* low thrombocyte counts (97—147,000) were accidentally found.

In December 1954 normal parturition. A few days later fever thought to be due to endometritis. In January *1955* fever and *salpingitis* was diagnosed. Despite treatment with antibiotics, persistent septi fever arthralgia with arthritis of elbow and knee joints, facial edema (with ut signs of renal failure), agony and disorientation, and signs f cardiac affection with pericarditic friction, tachycardia, gallop rhythm and ECG changes. The patient also had anemia (Hb down to 8.2 g per 100 ml), leucopenia (WBC repeatedly below 3000), high ESR (maximum 127 mm/1 hour), hypergammaglobulinemia (3.0 g per 100 ml), and posit LE-cell phenomenon.

During some weeks the patient was critically ill, but after institution of ACTH treatment the acute symptoms abated, and the gammaglobulin decreased to 1.5 g per 100 ml. Shortly after discharge, she experienced heavy loss of hair Later she developed slowly healing ulcers of both ankles. In *1956* another acute course with high fever arthralgia, bilateral pleuropneumonia, anemia and leucopenia, rise f gammaglobulin level (to 2.0 g per 100 ml), and positive LE-cell test. Enlargement of the heart on X ray and ECG changes were interpreted as signs of myocarditis. The patient again improved on corticosteroid treatment, and during follow-up until 1963 she had no severe acute episodes. But she had signs of chronic illness with recurring slight arthralgia, Raynaud phenomenon, leg ulcers difficult to cure, and decreased resistence to infections with sinuitis, otitis, suppurating mandibular dental cyst, and herpes zoster

Recurring attacks of arthritis with heart affection and elevated antistreptolysin titres were reported also in probands 3, 34 and 50 (group A) and in proband 60 (group B). Valvular heart disease (mitral stenosis) developed in proband 34 and necropsy of proband 60 showed histologic lesions compatible with previous rheumatic endocarditis.

Another 5 probands showed rheumatic valvular affection *post mortem*. mitral stenosis in 1 group A (number 12) and in 2 group B probands (numbers 76 and 81) mitral and aortic stenosis in 2 group A probands (numbers 11 and 54) In only 2 of these probands (numbers 11 and 54) had the valvular defects caused marked clinical symptoms (cardiac incompensation).

Thus, in all 4 (7 0 %) of probands A and 3 (5.8 %) of probands B showed evidence of chronic rheumatic endocarditis clinically or *post mortem*.

Antistreptolysin 0 titre had usually been determined at the same time as SSC-factor The distributions of the results obtained in the probands are given in Table 17 which includes all the values noted in the hospital records. Borderline (150—250 U) and positive (≥ 300 U) tests were numerically more common in group A probands. The difference in distribution of ASL titres between the two groups of probands was however not statistically significant (χ = 3.26, DF = 2, P > 0 10).

Positive ASL titres were not conceived

Table 17 Distribution, among negative (—), borderline (±) and positive (+) results, of test for ASL in probands. Compilation of previous results noted in the hospital records

Group[1]	Total tested	Total No. of tests	Test for ASL —	±	+
Probands A (57)	48	226	83	67	76
		%	36.7	29.7	33.6
Probands B (52)	47	166	6	42	48
		%	45.8	25.3	28.9

[1] Males and females pooled; all ages

as a manifestation of SLE and are therefore not included in Appendix Table I.

HEPATITIS

Hepatomegaly was reported in 9 (15.8 %) of group A and in 11 (21.2 %) of group B probands. Four (7.0 %) of group A and 6 (11.5 %) of group B probands had histologically verified *chronic hepatitis* or liver cirrhosis (these cases are marked with + in Appendix Table I). One proband A was examined with liver biopsy which showed slight, chronic hepatitis (proband 18). One male proband A had considerable enlargement of the liver and spleen for several years, he died from a bronchial carcinoma, but *post mortem* also showed liver cirrhosis and "diagnostic" signs of SLE.

Proband 14 (group A), male born 1899, in *19*[illegible] developed arthralgia, and *19*[illegible] polyarthritis with deformities was noticed. The ESR was then 65—82 mm 1 hour and the WBC 4000. A bout of purpura on the lower limbs was interpreted as "rheumatic" purpura. In *19*[illegible] for the first time the spleen was palpable and in *19*[illegible] for the first time also the liver.

In *19*[illegible] the patient was admitted because of fever splenomegaly and hepatomegaly. The liver was palpable down to the level of the umbilicum, the spleen was likewise markedly enlarged. The patient had persistent pigmentations of the legs after several previous spells of purpura. Other findings: leucopenia (minimum value 2100), thrombocytopenia (80—90,000), ESR 93 mm 1 hour, gammaglobulin 2.5 g per 100 ml, doubtfully positive LE-cell phenomenon.

In *19*[illegible] the patient had acute respiratory difficulties and was found to have paresis of the left recurrent nerve as well as laryngitis. He also had anemia (Hb about 10 g per 100 ml) and severe leucopenia (minimum value 920), hypergammaglobulinemia as before and typical LE cells in leucocyte smears.

In *19*[illegible] the liver and the spleen appeared to occupy the major part of the abdomen. The patient had cachectic habitus and pronounced drumstick fingers. Severe hemocytopenia. Hb about 7.5 g per 100 ml, WBC 300—700, thrombocytes 70—90,000. The LE-cell phenomenon was again positive. Bromsulphalein test: 13 % retention after 30 minutes. Liver biopsy showed nothing definitely pathologically. The patient was placed on corticosteroid treatment, which produced an extremely good subjective improvement with increase of bodyweight. At follow-up later in 1959 the liver and the spleen were enlarged as before but the BSP-test now showed 6 % retention, the gammaglobulin value had fallen to 1.[illegible] g per 100 ml, the WBC had increased to about 1000 and the Hb was normal. Another liver biopsy still showed no signs of cirrhosis. During *19*[illegible] however the patient had increasing cough and back pain. On hospitalization hypercalcemia was noted as well as increased NPN. After a few days in hospital he died in coma. Necropsy showed, unexpectedly, bronchial cancer with metastases of the liver and skeleton as well as nephrocalcinosis. The liver showed distinct microscopic signs of portal cirrhosis. The kidneys were reported to show preponderant interstitial nephritis, but at re-examination in 1963 (Prof C-G Ahlström) the "diagnostic" basal membrane changes were also seen.

On *post mortem* examination, additional 2 probands (both in group A) unexpectedly showed histologic signs of moderate portal cirrhosis (numbers 6 and 57).

Table 18. Sjögren's syndrome in probands

Manifestation	Probands A (57)		Probands B (52)	
	No.	%	No.	%
Keratoconj sicca only	1	1.7	5	9.6
Sialadenitis only	3	5.3	2	3.8
Keratoconj sicca *and* sialadenitis	2	3.5	9	17.3
Total	6	10.5	16	30.7

Of the 6 group B probands with signs of liver disease, 5 had been described previously by Krook (1961) as "liver cirrhosis in patients with a lupus erythematosus-like syndrome" They all had histologically verified liver cirrhosis. They also showed a varying degree of systemic involvement suggestive of SLE and were therefore included in the present group B It should be observed that 3 of these 5 patients had keratoconjunctivitis sicca and/or chronic sialadenitis. The 6th case in group B with liver disease also had chronic polyarthritis, Sjögren s syndrome and acrosclerosis:

Proband 71 (group B), female born 1911 Since *1941* periodically arthralgia and transient polyarthritis. In *1945* leucopenia during aurotherapy ESR then 25—74 mm/1 hour *1946*—1954 the patient was 4 times in department for surgical diseases because of parotid swelling with fever During the same tim some spells f purpura of the lower limbs. The ESR was always high, and in *1944* the SSC-test was positive (160). In 1960 the ESR was 112 mm/1 hour Raynaud phenomenon was noticed. Th patient complained of dryness of the eyes. In *1961* the ESR was still higher 143 mm/1 hour and electrophoresis (done for the first time) showed excessive hypergammaglobulinemia (3.0 g per 100 ml). The patient exhibited drumstick fingers, Raynaud phenomenon and acrosclerosis, and dyspnea on exertion without other signs of cardiac incompensation. The parotid and thyroid glands were somewhat enlarged and of increased consistency The WBC were somewhat low (3200). Two LE-cell preparations were positive. A liver biopsy showed chronic hepatitis with round cell infiltration and focal fibrinoid necrosis.

Nine probands A and 6 probands B had pathologically increased bromsulphalein retention or icterus ascribed to acute hepatitis. In none of these cases (marked with (+) in Appendix Table I) histologic liver studies had been performed.

SJÖGREN'S SYNDROME

The manifestations compatible with Sjögren s syndrome and seen in the probands are listed in Table 18. Owing to selection, such manifestations were more common in group B than in group A probands. A total of 16 (30 7 %) of the former and 6 (10.5 %) of the latter had one or both of the two characteristics, namely keratoconjunctivitis sicca and sialadenitis.

Of the 22 probands with keratoconjunctivitis sicca and/or sialadenitis 5 (1 in group A and 4 in group B) had chronic joint involvement. Nine probands (5 in group A and 4 in group B) had acute-subacute polyarthritis, while 5 (all belonging to group B) had arthralgia only and 3 (all in group B) had no joint symptoms at all.

The history of 1 of the 2 probands with "definite SLE having both characteristics of Sjögren s syndrome is given below

Proband 34 (group A), female born 1926. *1944*—1956 she was observed for uterine myoma and endometriosis, and the ESR then varied between 15 and 30 mm/1 hour In the summer of *1941* swelling was noted in the left parotid region. ESR 30 mm/1 hour Hb 10 g per 100 ml, WBC 2700. The swelling was interpreted as tumour It was irradiated and operated upon, but pathologic examination only showed damaged inflammatory tissue.

Three months later the patient developed joint

pain and joint swelling and high fever She was cared for in hospital for 4 months and then had polyarthritis, recurring bouts of f ver erythema on the tops of th fingers and on eyelids, signs of myocarditis with tachycardia and ECG changes, and bilateral basal roentgenographic lung changes. Increase of liver enzyme levels in the blood and bromsulphalein retention of 18 % after 30 minutes gave reason to suspect liver damage. Hematology: anemia with Hb down to 8 6 g per 100 ml, marked leucopenia with lowest value 1100 ESR increase to 86 mm/1 hour gammaglobulin t 2.6 g per 100 ml. Repeated examinations for LE-cells positive. SSC-test positive (256). The ophthalmologist diagnosed keratoconjunctivitis sicca. The patient gradually improved on corticosteroid therapy

It should be noted that the figures giv en above for the incidence of keratocon junctivitis sicca and sialadenitis, respec tively probably do not represent the real incidences, since only a minority of the probands had been examined by ophthalmologists or questioned specifically for xerostomia and parotid swelling. It is remarkable that these manifestations were found also in patients without any joint symptoms or with arthralgia only

No data on the incidence of Sjögren s syndrome in SLE have appeared as yet, although the constellation of the two syndromes has been recognized (see Chapter 1).

HEMOLYTIC ANEMIA

Ten probands A had anemia combined with a positive direct antiglobulin (Coombs') test. Four of them also had reti culocytosis making the diagnosis of hemolytic anemia probable; the other 6 Coombs positive probands were not investigated sufficiently for hemolysis. In 3 cases, Coombs positive anemia wa combined with thrombocytopenic purpura (probands 9 42 and 56). The picture was dominated by hemolytic anemia in th follow ing case:

Proband 33 (group A), female born 1907 Since *1950* periodic pain and swelling of the joints of the extremities. High ESR was known since 1957 (80 mm/1 hour)—in 1958 it had increased t 126 mm/1 hour After 1 month phenylbutazone therapy anemia was noticed for th first time (Hb 8.0 g per 100 ml, RBC 2.6) in September *1958* Despit withdrawal of the drug the anemia gradually deepened, and in January *1959* the Hb was 6.3 g per 100 ml, and the RBC 1.9 million per cmm. Reticulocytes constituted 10 % of the RBC, the direct antiglobulin (Coombs') test was repeatedly positive and the haptoglobin level was low (10 mg per 100 ml). The Wassermann's, Meinicke's and Kahn' reactions were positive but the TPI negative. The SSC-test was positive (128). The gammaglobulin was 1.8 g per 100 ml, and the LE-cell test was positive. The patient was treated with corticosteroids which resulted in steady increase of the red blood values. During the following 3 years steroid treatment was continued and the patient condition was good with normal RBC, ESR near normal, absence of joint complaints, but there was a tendency to slight arterial hypertension.

In proband group B no positive antiglobulin tests were reported, but one female had a hemolytic crisis preceding other manifestations of collagen disease by several years:

Proband 70 (group B), female born 1892, was ill in *1948* with fatigue, jaundice and fever Hemolytic anemia was diagnosed. RBC down to 1.3 million per cmm, Hb down t 4.9 g per 100 ml, slight increase of serum bilirubin, and lowered osmotic resistance of the erythrocytes. The patient got blood transfusions and gradually improved. During the following years she was troubled by symptoms of chronic bronchitis, but the blood values were normal.

In *1955*, symptoms and signs of polyarthritis developed. Chest X-ray: signs of previous bilateral pleuritis. Electrophoresis showed hypergammaglobulinemia (1.3—1.9 g per 100 ml), and the LE-cell phenomenon was repeatedly positive. In *1959* the patient was severely troubled by arthralgia but showed only minimal objective joint changes. She appeared chronically ill and had recurrent fever Systemic sclerosis was suspected because of focal periarticular calcification with surrounding sclerosis on histologic examination, decreased peristalsis on X-ray of the esophagus, and streaky infiltrations of both lung bases on chest X-ray Signs of nephritis were bserved, renal insufficiency developed, and the patient died in uremia. *Post mortem* was incomplet The kidney were said to show "nephrosclerosis The liver was said to contain focal leucocyte infiltration and necrosis.—It was not possible to procure the pathologic specimens for re-examination for the present study

Antibodies to red cells were thus demonstrated in 10 probands with definite

SLE, and 1 proband with suspected SLE had a severe transient hemolytic anemia. The real incidence of antibodies to blood cells cannot be estimated, since in most patients Coombs test had not been done. The same statement applies to the SLE series in the literature

THROMBOCYTOPENIC PURPURA

Thrombocyte counts less than 1.0 000 were reported in 18 (31 6 %) of group A and in 9 (17 3 %) of group B probands. Of these, 5 probands A and 2 probands B had co-existing purpura. In 2 cases (probands 42 and 56, group A) the thrombocytopenic purpura was the dominating feature of the SLE syndrome.

Proband 44 (group A), female born 1924. In *1943* transient m trorrhagia, thrombocytes not examined. Since *1944* slight arthralgia and Ray naud' phenomenon. In June, *1957* first appear ance of purpura, and on the same time pronounc ed menorrhagia. Thrombocyt counts below 50,000 lowest 26,000 per cmm. Prolonged bleeding time: 14 minutes. Hb down to 6.6 g per 100 ml, reticulocytes 16 % of RBC, Coombs' direct test positive. ESR 50 mm/1 hour gam maglobulin 1 6 g per 100 ml, LE-cell test posi tive. Moderat swelling of the joints of the fingers and of the right knee were noticed. Treatment with corticosteroids led to cessation of purpura, but thrombocytopenia persisted.

Three months later in October 1957 again abundant uterin bleeding. Thrombocytes 23—24,000. The bleedings were controlled by increase of corticosteroid dosage. In February *1958* the thrombocytes were 58—72,000 and in April 210,000. In June 1958 were red infiltrations of the finger tops noticed, consistent with LE. Slight arthralgia as before. No bleeding manifestations. In August, *1961* th corticosteroids were withdrawn. In September 1961 still no complications, ESR 9 mm/1 hour thrombocytes 248,000.

INCREASED ERYTHROCYTE *SEDIMENTATION RATE*

All the probands had an increased ESR (more than 20 mm/1 hour) some time during the course of the illness. It often exceeded 100 mm/1 hour during the acute attacks and many probands had a *chronically high ESR.* Pathologic ESR values had *often* been observed together with initial subjective symptoms and had then usually persisted, other manifestations of SLE gradually supervening (Table 14)

Proband 21 (group A), female born 1923. In *1951* the patient was dmitted to the department of psychiatry because of reactive depression. Increased ESR (20—45 mm/1 hour) was then observed. A left-sided ovarian cyst was found and was removed at operation, but the high ESR persisted. Some low WBC were also noted (minimum 2900). During pregnancy in *1954* slight proteinuria. After parturition the patie t was treated with blood transfusion because of uterine bleeding but sh reacted with chills.

From *1955* the patient had recurrent moderately painful swelling of both parotid glands, arthralgia and attacks of cyanosis of the fingers on exposure t the cold. In *1960* th cause of the high ESR (50—80 mm/1 hour) was again considered. Th patient was treated with penicillin because of vaginal leucorrhea but the high ESR persisted. Electrophoresis showed elevation of the gammaglobulin to 2.8 g per 100 ml. In *1961* re-investigation because of the increased ESR (72 mm/1 hour)· no objective joint changes, somewhat enlarged and tender submandibular salivary glands, moderat leucopenia (lowest value 2900), gammaglobulin 2.5 g per 100 ml, strongly positive SSC-test (256) but 3 examinations for LE-cells negative.

After the patient had left hospital shortness of breath, stabbing pain in th left of the chest and swelling of the fingers occurred. She was re-admitted with a diagnostic episode of fever left sided pleurisy purpura of the legs, subacute butterfly exanthema, and positive LE-cell phenomenon.

ESR was not registered as a manifestation of SLE in Table I, Appendix, because it may be conceived as an expression of serum protein changes which can be stud ied in greater detail by electrophoretic methods.

Most authors report an increased ESR in almost 100 % of SLE series. An ele vated ESR as an *early* and *chronic* sign of SLE (Larsson and Leonhardt 1959) seems often to have escaped attention.

HYPERGLOBULINEMIA

The serum protein pattern had been analysed by electrophoresis on paper in all

Table 19 Distribution of gammaglobulin values of probands. Compilation of previous results noted in the hospital records

Group[1]	Total examined	Total No. of examinations	Gammaglobulin[2]			
			<1.0	1.0—1.4	1.5—1.9	≥2.0
Probands A (57)	50	167	9	30	45	83
		%	5.4	18.0	26.9	49.7
Probands B (62)	45	166	3	16	46	101
		%	1.8	9.7	27.7	60.8

[1] Males and females pooled, all ages
[2] The gammaglobulin values are given in g per 100 ml in this and in all subsequent tables

but 5 probands (1 of group A and 4 of group B).

Gammaglobulin values of 1.5 g per 100 ml or higher on at least occasion had been found in 90 % of the probands of both groups (Table 19). In 39 (68.4 %) of probands A and in 36 (69.2 %) of probands B, values of 2.0 g per 100 ml or higher had been recorded at least once. In only 4 of the probands examined electrophoretically had consistently low gammaglobulin values been noted. Three of them (probands 66, 110 and 123) had however nephrosis with low total protein values; in the 4th (proband 90), only a single determination had been made, and the total serum protein value was then 5.2.

Of the 7 probands who had not been studied with electrophoresis, 6 had had their serum proteins examined by salting out methods. Hyperglobulinemia had thereby been noted in 4.

Hyperglobulinemia, as noted by electrophoretic or by precipitation methods, had often been present for a considerable time before the diagnosis (Table 14). These patients also had chronically high ESR.

Proband 89 (group A), female born 1923, fell ill in *1954* with fatigue dizziness and anorexia. Anemia with Hb 9.4 g per 100 ml and ESR of 91 mm/1 hour were noted, but X-ray of the stomach and biliary system showed nothing remarkable. The high ESR persisted, and later in 1955 electrophoresis was done showing gammaglobulin value of 2.2 g per 100 ml. The thymol turbidity reaction was also strongly positive. Hepatitis was assumed but the bilirubin in serum was not increased. A tendency to leucopenia was noted with lowest value 2400. Right sided pupillotonia was accidentally found. The elevation of the ESR and the increased thymol turbidity reaction were persistent at later follow-up.

In *1957* pain and swelling of the joints of the limbs supervened in association with sore throat and fever. In *1958* severe arthralgia, but only slight objective signs of joint involvement. The SSC-test was, however positive (64, 128), the ESR was about 100 mm/1 hour the gammaglobulin level was on 2.2 g per 100 ml, and the blood cell counts were low (RBC 2.8, WBC 2000 and thrombocytes 102,000 at lowest). The patient received aurotherapy without complications, but later in 1958 she had fever and severe urticaria with Quincke-like edema of the face during treatment with phenylbutazone. She also reacted with exanthema during penicillin therapy. At the end of 1958 bilateral enlargement of the sublingual glands was noticed, as well as bilateral pupillotonia. LE-cell test, performed for the first time was negative.

In *1961* the patient exhibited clear-cut symmetric polyarthritis. During spell in hospital because of the joint affection, she had bouts of fever pleurisy pericarditis, roentgenographic enlargement of the spleen, and transient exanthema. Hematology: Hb down to 8.0 g per 100 ml with positive Coombs direct test but without reticulocytosis, WBC down to 1300 thrombocyte counts often below 150 000. Liver involvement was suspected because of glutamic-oxalacetic transaminase increase (60 U), but the bromsulphalein test showed only 3 % retention after 30 minutes. The transaminase elevation may have been due to myositis—muscle biopsy showed slight chronic interstitial myositis. The gammaglobulin level was on 2.3 g per 100 ml. The diagnosis of SLE was confirmed by repeated demonstration of typical LE-cells.

Proband 41 (group A), female born 1888. Healthy until *1944* when she sought advice for fatigue. Examination revealed ESR 96 mm/1 hour. This prompted extensive examination for malignant disease but no explanation for the high ESR was found. T tal serum protei was recorded as 9 6 and globulin as 4.6 g per 100 ml (salting-out method).

In *1948* the patient had "erysipelas of the face with high fever but the rash persisted for 3 months. In *1950* re-investigation because of persistently increased ESR (about 100 mm/1 hour). The thymol turbidity zinc sulphate and formol gel reacti ns were strongly positive. No signs of liver disease, bromsulphalein test negative. Slight anemia (Hb about 10 g per 100 ml) and leucopenia (WBC 2600—4000). Extensive roentgenologic examination, slight enlargement of the spleen but therwise nothing remarkable. During stay in hospital the patient had transient phlebitis of the left thigh.

1950 and 1951 attacks of right-sided pneumonia. In *1951* slight enlargement of both pleen and liver was palpated. Blood transfusion because of nemia (Hb 8 g per 100 ml) caused chills and fever. The patient had a few other spells of fever without obvious cause; signs f impaired renal functio in the form of isosthenuria and tendency to acidosis and leucopenia with lowest value 1100. The Wassermann s reaction was positive and was thought to be non-specific. Later in 1951, typical butterfly exanthema of the face developed, and also rash on the upper part f the chest. Slight proteinuria and slightly elevated NPN were noted, but the urinary sedi ment was repeatedly normal.

I *1953* acute bronchitis. During stay in hospital the patient also exhibited transient red infiltration of the right cheek, interpreted as CDLE. ESR was increased up to 141 mm/1 hour T tal protein 8.0 and globulin 4 0 g per 100 ml. Lowest Hb 8.2 g per 100 ml, WBC 2700, thrombocytes 141 000. Traces of protein in the urine NPN 28—50 mg per 100 ml, urinary sediment normal. In *1954* paper electrophoresis was performed for the first time. A gammaglobulin value of 4.1 g per 100 ml was then recorded.

The following years the collagen disease was quiescent but advancing senile demens was noted. On check examination in *1959* excessive hypergammaglobulinemia was th most prominent finding: gammaglobulin 3.8 g per 100 ml. Hb 10.4 ml, WBC 3600, thrombocytes 126,000 Slight proteinuria. No actual skin lesions. In *1960* th patient died in her home from "general debility Necropsy was not performed.

Illustrating figure: see Waldenström (1962).

In Chapter 2 it was pointed out that the finding of hypergammaglobulinemia had often led to the suspicion of SLE in the primary material and that this might have increased the number of selected probands with hypergammaglobulinemia (see page 28) In the published series of SLE, *hyper globulinemia* was reported in 28—73 % (Table page 11) However only a minor ity of the patients in these materials had been investigated by electrophoresis. Therefore, it cannot be decided if the incidence of hyper*gamma*globulinemia is *de facto* higher in the present series than in the literature series.

LE-CELL PHENOMENON

Forty three (75 4 %) of group A and 28 (53.8 %) of group B probands had one or more positive LE-cell tests.

A few of the present cases showed a positive LE-cell phenomenon for the first time several years after the onset of symptoms and in a few cases no LE-cells were ever found, despite repeated examination, but the diagnosis of SLE was confirmed post mortem.

Proband 84 (group A), female born 1935. At 20 years (1955) th patient developed widespread CDLE. Sh also had recurrent fever spells of arthritis, anemia and leucopenia, ESR ver 100 mm/1 hour hypergammaglobulinemia, and signs of nephritis with arterial hypertension. Sh died after only 10 months' illness, and necropsy show ed the "diagnostic changes of the spleen and of th kidneys. During the course, LE-cell tests had been done on 10 occasions but were always negative.

The incidence of positive LE-cell phenomenon in large SLE materials has been described as varying from 69 to 96 % (see Table 2). The LE-cell phenomenon was obviously often conceived as an almost obligatory criterion. If the diagnosis of SLE be accepted in clinically suspected cases in the absence of positive tests for LE-cells, the incidence will be much lower as pointed out by Rothfield *et al* (1963)

As a comment on the *specificity* of the LE-cell phenomenon, a reference might be made to Table 6 (page 27). Of the 99 primary cases with a positive LE-cell phenomenon 27 were excluded from the

proband series. Two of the exclusions were patients with SLE, each of whom had a sister with SLE already included. Of the other 25 11 were patients with rheumatoid arthritis, 5 had drug hypersensiti ity states, 1 had polyarteritis nodosa and 2 undiagnosed vague illness reminiscent of SLE. Only 6 had a disease not commonly associated with SLE, namely sarcoidosis. In this disease, however a component of autoimmunity has also been suggested (see Mackay and Burnet 1963). The other 72 primary cases with positive LE-cell phenomenon had a history consistent with SLE and were included in the proband series. Thus, the usefulness of the LE-cell phenomenon as a differential diagnostic tool was demonstrated also in the present investigation.

FALSE-POSITIVE SEROLOGIC TEST FOR SYPHILIS

All the probands had been examined on one r more occasions with one or more tests (Wassermann, Meinicke, Kline and Kahn) for syphilis. In 9 (16 8 %) probands of group A and in 8 (15.4 %) of group B one or more of the tests were positive usually in low titer without clinical evidence f syphilis. The TPI test was performed on the sera of 6 probands with a positi e STS (4 in group A and 2 in group B) and was then found t be negative.

In addition, serum from 9 probands of group A and 4 from group B were reported to be anticomplementary These results are given as STS, false-positive (+) in Appendix Table I.

In none f the cases was a false-positive serologic test fo syphilis an initial sign, but in some cases positive reaction was noted several years before the diagnosis of SLE was established (g probands 8 and 9 see also Table 14)

SCLERODERMA AND MYOSITIS

Attacks of peripheral cyanosis on exposure t cold (Raynaud ph nomenon) were often described in the probands: in 13 (25.0 %) of group A and in 11 (21.2 %) of group B probands.

The probands with Raynaud-like symptoms were often said to have some atrophy of the skin of the extremities, especially of the fingers, but these findings were usually concomitant with joint deformities. In 4 probands (all of group B) the changes were so pronounced that they were labelled as *acrosclerosis* however skin biopsy was not performed. In 1 of these subcutaneous calcification and decreased peristalsis of the esophagus were also detected on X ray (proband 70 page 51).

In still another case changes of the esophagus were demonstrated and disabled the patient severely in another 2 cases (proband A) subcutaneous calcifications were reported.

Proband 9 (group A), female born 1906. In *1940* the patient developed arthralgia, and examination in *1941* disclosed swelling f the proximal interphalangeal joint bilaterally and of the right ankle, ESR 33—110 mm/1 hour and repeatedly positive Kahn reaction. The pain gradually abated but returned in *1944*. In *1945* the patient had acute abdominal pain and diarrhea and was operated on for suspected gynecologic disease. But the operation revealed that the lowermost 30—40 cm of the ileum was cyanotic and edematous. After operation the patient had fever bilateral pleuropneumonia, facial edema as well as widespread dermatitis and st matitis. Laboratory studies: Hb 9 6 g per 100 ml, RBC 2.4 million per cmm, WBC 2100 (minimum values recorded). Traces of protein in the urine. ESR 32—63 mm/1 hour The acute condition gradually abated (without corticosteroid treatment), but the ESR increase persisted. In *1946* the serum proteins were evaluated for the first time: total protein 7.3 and globulin 4.1 g per 100 ml (salting-out method).

I *1947* bilateral pleurisy returned, and the patient showed sloughing facial exanthema, conceived as seborrhea Hb 9—11 g per 100 ml, RBC 3.5—4.2 million and WBC about 3500 per cmm. ESR 63—92 mm/1 hour thymol turbidity test positive. Wassermann self inhibiting, Kahn test weakly positiv Roentgen examination: slight splenomegaly

The following years good general condition but spells of arthralgia and rash of the face on exposure t sunshine. The ESR was persistently increased, there was tendency to leucopenia, and positive Kahn reaction was noted on several occasions. In *1951* slight transient jaun-

dice, which was believed to be due t gallstone [illegible] dermatologist diagnosed the facial erythema as CDLE and instituted treatment with bismuth injections. These were followed by severe arthralgia and fever and subacut polyarthritis, stomatitis, and a spell of purpura, mainly of the legs, were noted. She also had ECG signs of myocarditis, including transient auricular fibrillation. During blood transfusion the patient went into shock with tachycardia, followed by fever Blood examinations disclosed pronounced hemocytopenia (RBC down to 2.1 million per cmm, WBC repeatedly below 2000 and thrombocytes below 50,000 per cmm). Roentgenography still showed enlargement of the spleen. Thymol turbidity was strongly positive Examinations for LE-cells proved negative. The patient was treated *inter alia* with cortisone and improved after 4 months of acut illness.

At follow-up in *1953* the ESR was still about 100 mm/1 hour the hemocytopenia persisted, and roentgen examination still showed lamellar atelectasis in the base of both lungs as well as splenomegaly A positive direct Coombs' test was recorded, and examination for LE-cells proved positive In *1954* th patient developed increasing swallowing difficulties. Roentgenography showed stricture of the esophagus (see Figure 1).

Since 1954 the patient was treated continually with cortisone. Despite this, she had spells of severe arthralgia, pronounced swallowing difficulties and persistent rash of the face. X-ray in *1956* showed, as before, stricture f the esophagus. In *1958* the patient still had high ESR, anemia and leucopenia, positive Meinick and Kahn's reactions, and positive LE-cell phenomenon. Butterfly exanthema of the face improved after treatment with antimalarial drugs.

During the following years the patient's condition gradually improved. Th blood values and the ESR returned to near normal. However in *1961* the patient had bilateral bronchopneumonia, recurrence of facial exanthema, and ascites was noticed for the first time. She responded t treatment with antibiotics, corticosteroids, antimalarial drugs and diuretics, respectively and under circumstances she was then doing fairly well.

Myalgia was a common complaint but was usually masked by the joint symptoms. A muscle biopsy was performed in proband 29 (group A) and showed interstitial myositis. On *post mortem* another patient was described to have changes consistent with *polymyositis*

Proband [illegible] (group B), female born 1915. In *[illegible]* arthralgia concomitantly with exanthema interpreted as rubeola. In *[illegible]* joint affection



Figure 1 Stricture of esophagus in proband 9

progressed t disabling polyarthritis. During phenylbutazone therapy WBC decreased from 6300 to 2000. The patient also had moderate anemia (Hb 9.1 g per 100 ml), ESR 65—95 gammaglobulin 1 7 g per 100 ml, but the test for LE-cells was negative. The SSC test was borderline.

In 1957 the patient received urotherapy b t reacted with exanthema. I 1960 she had sudden attack of hematemesis, and X-ray showed esophageal varices. The spleen was palpabl down t the level of the umbilicus, there was secondary anemia, severe leucopenia (WBC 300—1500), and thrombocytopenia (74—90,000). The gammaglobulin level had risen to 3.5 g per 100 ml, positive LE-cell phenomenon was now demonstrable, and SSC-test was strongly positive (1024). Bromsulphalein retention 9 % after 30 minutes. Because of recurrent hematemesis, the patient was submitted t laparot my for portal-caval shunt. On operation, however the



Figure 2. Myositis in proband 63 (*post mortem* specimen).

portal venous pressure was found t be normal therefore no shunt was established, but the spleen was removed. Histologic examination of the spleen showed stasis and no signs of SLE. Postoperatively the patient had short bouts of fever and transient uricular fibrillation. She also showed marked periungual erythema bilaterally But the blood alues rose t normal levels.

A few months after operation the polyarthritis was much worse The patient also complained of poor general muscle strength, and the muscles of the limbs were severely atrophic. She had new bouts of f ver varying pleuropneumonic hanges, and attacks of supraventricular tachycardia The LE-cell phenomenon was again positive One ear later the patient had increasing dyspnoea and was admitted because of acut respiratory difficulties. She died during tracheotomy *Post mortem* showed polyserositis, chronic interstitial myocarditis, multiple arteritis and small interstitial granulomata of the lungs, few hyaline glomeruli in the kidneys but no "wire-loop lesions. In muscle specimens (psoas and diaphragma), heavy and widespread interstitial infiltration of inflammatory cells and slight t moderat degeneration of muscle fibres were noted, the pathologist diagnosis was "severe hronic polymyositis (Figure 2). On re-examination of the specimens in 1963 (Prof F Linell) the findings were confirmed, besides which sclerotic hanges and round cell infiltrates in the corium, consistent with scleroderma, were observed.

THYROIDITIS

Slight to moderate thyroid enlargement was reported in 7 (12.3 %) of group A and in 6 (11.5 %) of group B probands. None of these cases had had any apparent thyroid dysfunction. One proband of group A had been operated upon because of progressive painful thyroid enlargement. The pathologic diagnosis was Riedel s thyroiditis. In retrospect, however these changes might be interpreted as the final stage of a Hashimoto s thyroiditis.

Proband 47 (group A), female born 1913. After parturition in *1942* recurrent abscesses of the mammae. Following this, first appearance of arthralgia, with transient swelling of hand and foot joints. In *1945* operation because of extrauterine gravidity Postoperatively pneumonia and, 3 weeks later arthralgia again. Since 1945 also increasing thyroid enlargement. Tw sisters and maternal aunt were said to have been operated upon because of goitre. In *1953* thyroidectomy was performed. Histologic diagnosis: Riedel-thyroiditis.

Mean hile, chronic progressive polyarthritis had developed. In 1946 rheumatic noduli were noted for the first time. After parturition in 1950 exacerbation of arthritis. Aurotherapy in the same year was accompanied by eosinophilia. In *1955* pneumonia and obstinat ulcers of both legs. I *1958* facial edema developed. Nephropathy was diagnosed—proteinuria, hematuria, moderate arterial hypertension. Gammaglobulin 2.7 g per 100 ml. Waaserman's test, self-inhibitory effect. In *1961* exanthema in conjunction with treatment with antimalarials. Increasing hypertension, signs of cardiac incompensation. Death in uremia. *Post mortem* showed focal necrotizing glomerulitis, signs of hypertension and uremia, and in muscle preparations fibrinoid necrosis and lamellar fibrosis of small arteries were noted. Re-investigation of the thyroid preparation from 1953 (Prof C-G Ahlström, 1963) showed chronic fibrotic changes consistent with Riedel thyroiditis.

One case of probable Hashimoto s thyroiditis was included in group B because f ther collagen symptoms (proband 108).

The other cases with thyroid enlargement had not been investigated specifically as t Hashimot s thyroiditis. However this form f thyroiditis was found ccidentally at a topsy f 1 proband of group B In this woman no thyroid enlar

gement had been noted. She also had Sjögren's syndrome and purpura of Waldenström's type:

Proband 61 (group B), female born 1885. The earlier history included pneumonia after parturition in 1919, cholecystectomy in 1924 and operation for uterin prolapse in 1937. The patient reported that the ESR had continually been above 70 mm/1 hour since her last operation. In *1944* the ESR was recorded as 84 mm/1 hour. In *1947* when treated for left-sided pleuropneumonia, the patient had ESR 65—115 mm/1 hour and hyperglobulinemia (total protein 8.8, globulin 4.4 g per 100 ml—salting-out method). In 1945 and 1947 the WBC were rather low (2500—3200). *1945* the patient had "tumour of the left parotid gland" but biopsy showed chronic sialadenitis. *1951* keratoconjunctivitis sicca was diagnosed by the ophthalmologist and purpura hyperglobulinemica by the internist (Prof J Waldenström). The patient also had history of Raynaud' phenomenon.

Decreased resistance to infections led to bronchopneumonia and sinuitis in 1951 otitis and bronchopneumonia in 1952. The WBC were low even during infections (2000—3000). In 1953 the patient developed myalgia and in *1954* she had peripheral symmetric polyarthritis with erosions of the small joints of the hands on X-ray rheumatic noduli, and a positive SSC-test. Electrophoresis showed a gammaglobulin value of 3.5 g per 100 ml, and the LE-cell phenomenon was positive.

In 1954 the patient had obscure attacks of abdominal pain and in 1955 recurrent attacks of loss of consciousness interpreted as Adams-Stokes' syndrome. In *1956* paraplegia of unknown origin occurred. The patient died later in 1956. *Post mortem* revealed remarkably slight arteriosclerosis, but the spleen was moderately enlarged, and the thyroid (which was of normal size) showed pronounced changes with lymphoid and plasma cell infiltrates. The diagnosis of Hashimoto's thyroiditis was verified on check-examination (Prof F Linell) in 1963, when also angiitis with plasma cell infiltration was seen in the intestinal mucosa and in the perirenal tissues.

POST MORTEM FINDINGS

All 19 probands A and 16 of 18 probands B who had died during the period of selection (1955—1961) had been examined *post mortem*. All patients with "diagnostic" autopsy findings had been referred to group A. Libman-Sachs' endocarditis was described in 3 (15.8 %) of the 19 probands A examined, pronounced periarterial fibrosis in the spleen in 16 (84.2 %) "wire-loop" lesions and/or focal necrosis of the glomerular tufts in all 19 (100 %). Hematoxylin bodies were not described in the necropsy records or seen at re-investigation.

Besides the "diagnostic" morbid pathologic lesions, other changes consistent with the diagnosis of SLE were often described in both groups of probands, including myocarditis, polyserositis, glomerulonephritis, and arteritis. Other findings of special interest are referred to in the case reports.

In spite of a "classic" history of SLE, necropsy in some cases revealed only unspecific changes (*e g* proband 60 page 60).

ETIOLOGIC FACTORS

INFECTIONS

In the present series of probands, as in other SLE series, infectious complications, such as bronchopneumonia otitis, sinuitis and urinary tract infection were common. One patient (proband 66) was found to have miliary tuberculosis *post mortem*, probably owing to heavy steroid therapy and one had tuberculous foci in the abdominal cavity. These were, however the only cases in which active tuberculosis was demonstrated.

The probands of group A as well as of group B often reported that the acute attacks had been preceded by infections, usually of the respiratory tract. As a rule, it was difficult to check these anamnestic data which are therefore of little value from an etiologic point of view.

In some cases the infections and the other manifestations of the disease were apparently intimately related.

Proband 1 (group A), female born 1917. In *1939* the patient had scarlatina, followed by

spell of polyarthritis. ESR was then 35 mm/1 hour and never afterwards reached normal values—it was usually above 40 mm/1 hour. The joint pain recurred almost annually in association with tonsillitis until 1947 when tonsillectomy was done—after which she only occasionally had slight arthralgia. Since *1944* the patient had recurrent sinusitis, swelling of both parotid glands with xerostomia, and irritation of the eyes. In *1951* the ESR was about 100 mm/1 hour there was general enlargement of the superficial lymph nodes, both parotid glands were swollen, and keratoconjunctivitis sicca was diagnosed with the aid of Rose Bengal staining and Schirmer's test. Biopsy of the cervical and axillary lymph nodes showed non-specific inflammation with abundant deposition of plasma-cells. Laboratory studies showed hyperglobulinemia (total protein 8.6 and globulin 5 7 g per 100 ml—salting-out method), slight anemia (Hb about 11 g per 100 ml) and leucopenia (WBC 3200—4200).

Decreased resistance to infections was manifested in the form of sinusitis and bronchopneumonia in 1951, and submandibular abscess, sinusitis and bilateral bronchopneumonia in *1952*. She also had attacks of fever which responded to cortisone but not to antibiotics, varying degrees of anemia, leucopenia, and thrombocytopenia with purpura of the skin. When given blood transfusions she reacted with chills, stabbing pain in the chest and fever.

In *1953* the patient again had sinusitis and, on 2 occasions, pneumonia. By the end of 1953 her general condition was poor: she had mucosal hemorrhages in the oral cavity, intestine and urinary bladder and signs of myocarditis with pulmonary stasis. She also had a few epileptiform attacks despite normal NPN. During the first months of *1954* she was still in a critical condition. Repeated chest X-ray showed varying bilateral pleural and pulmonary changes. Laboratory studies showed pronounced anemia with Hb 6.4—8 8 g per 100 ml, RBC 2.0—2.5 million per cmm and WBC down to 800. Gammaglobulin 4 7 g per 100 ml. Early in 1954 typical cutaneous LE appeared with lesions in the face, on the elbow and the fingers. Cortisone treatment gradually produced improvement of the patient.

Fatigue and diffuse arthralgia as well as a tendency to eczema-like changes in the skin, however persisted. The patient also had severe loss of hair. Thrombocytopenic purpura again appeared. LE-cell test was made for the first time in autumn 1954 and was positive. Of 10 tests for ASL made during 1952—1955, all but 1 showed positive titres. In *1955* signs of renal disease were added with proteinuria, moderately increased NPN and edema. During her last spell in hospital in 1955 the patient had again several attacks of fever. Anemia and leucopenia persisted as well as the signs of nephropathy. The gammaglobulin level was about 4 0 g per 100 ml as before. An acute hemoptysis was the final cause of death in autumn, 1955. *Post mortem* showed acute erosive gastritis; polyserositis; concentric periarterial fibrosis in the spleen as well as "wire-loop" lesions in the kidneys.

ENDOCRINE FACTORS

That the sex distribution showed a preponderance of females has already been mentioned. SLE was diagnosed in fertile age in 76.4 % of group A female probands and 54.2 % of group B female probands. In many cases in which the diagnosis of SLE was not made until the menopause the patients had had manifestations already in fertile age.

In 2 cases the SLE syndrome made its first and acute appearance following gynecologic states, namely normal parturition in one (proband 10) and abortion in another (proband 42). Both patients had, however, had symptoms suggestive of SLE previously.

Proband 10 (group A), female born 1930. In *1953* first appearance of arthralgia and half a year later exanthema of the face, scalp and fingers. This was diagnosed as CDLE by the dermatologists. ESR 10—66 ml/1 hour, slight leucopenia (WBC 3100—5200), thymol turbidity test slightly positive. Proteinuria was occasionally noticed.

In July *1954* the patient had an abortion (mens III) which was followed after 1 week by septic type of fever, right-sided pleuritis, and transient circulatory shock possibly due to myocarditis. Infection could not be demonstrated. A few LE-cells were seen in leucocyte smear. The acute course abated on corticosteroid treatment. The arthralgia persisted, however and the patient showed signs of Raynaud' phenomenon.

In June, *1955*, a new severe exacerbation of SLE with varying cutaneous manifestations including heavy loss of hair, stomatitis and conjunctivitis; recurrent high fever, roentgenologic signs of bilateral pleuritis, ECG changes consistent with myocarditis, and severe psychosis with hallucinations. Also proteinuria with occasional red cells in the sediment but normal NPN. Hematology: anemia with Hb down to 6.4 g per 100 ml and RBC down to 2.1 million per cmm, Coombs direct test positive, leucopenia with lowest value 1100. Gammaglobulin level at highest 1.9 g per 100 ml. LE-cell test positive. Persistent slight proteinuria. The patient was severely ill during this course and spent 8 months in hospital

Tabl 20. Hypersensitivity reactions in probands

Type of reaction	No. of probands A with reaction			No. of probands B with reaction		
	Before diagnosis	*After* diagnosis	Total	*Before* diagnosis	*After* diagnosis	Total
Bronchial asthma	1	0	1	1 (+1)	0	1 (+1)
Allergic rhinitis	1 (+1)	0	1 (+1)	0	0	0
Eczema	1(+4)	0	1 (+4)	0 (+2)	0	0 (+2)
Angioneurotic edema	3 (+1)	0	3 (+1)	0	0	0
Urticaria (? food)	5 (+2)	1	6 (+2)	2 (+1)	1	3 (+1)
Drug reaction						
Penicillin	1	5	6	1	2	3
Sulfonamides	2	2	4	2	0	2
Gold	1	0	1	1	0	1
Antimalarial compounds	1	1	2	0	0	0
Phenylbutazon	6	3	9	5	2	7
Others	5	3	8	2	1	3
Transfusion reaction	5	1	6	0	2	2
Total hypersensitivity reactions	32 (+8)	16	48 (+8)	14 (+4)	8	22 (+4)

Numbers in parenthesis: only anamnestic information

but at last improved during corticosteroid therapy

During the following years the patient had changing arthralgia. She exhibited pronounced Raynaud phenomenon, and in *1959* necrosis of the top of one finger was bserved. Gradually the patient's arterial blood pressure rose, and in *1961* the patient had manifest hypertension secondary to chronic nephritis.

In at least one case (proband 27 see page 48) the exacerbation might be interpreted as a possible consequence of an *infection* post partum so that the endocrine factors may be of subordinate importance.

In another patient an early manifestation appeared, namely joint symptoms in association with abortion (proband 42).

HYPERSENSITIVITY REACTIONS

Table 20 gives the occurrence of different types of hypersensitivity in probands, e g asthma, hay fever urticaria and drug reactions (exanthema, bouts of fever leucopenia and other manifestations in immediate association with drug treatment). In the latter case it was sometimes difficult to decide which drug or drugs should be regarded as responsible for the reaction. The table includes only that drug which was most strongly suspected in a given case. Hypersensitivity not observed by a physician is given in the table in parenthesis.

In all 32 (56.1 %) probands A and 15 (28.8 %) probands B showed hypersensitivity reactions. Of the different types of hypersensitivity it was the *reaction to drugs* that was most striking. Twelve of the patients were probably hypersensitive to more than one substance and a few patients showed considerable intolerance to several drugs.

Proband 44 (group B), female born 1911 In 1948—1951 the patient was treated in hospital repeatedly for "recurring rheumatic fever She ultimat ly developed chronic polyarthritis but only slight deformities. Other manifestations noted were slight enlargement of reticulo-endo-

thelial organs (lymph nodes, liver spleen), recurrent pleuritis, pericarditis, septic fever anemia and leucopenia, positive STS, proteinuria and hematuria, hypergammaglobulinemia (1 7—2.0 g per 100 ml), and typical butterfly exanthema. She also had very pronounced hypersensitivity to drugs and reacted with urticaria and other types of exanthema, fever or leucopenia on treatment with gold, penicillin, tetracycline, chloromycetine, phenylbutazone and antihistaminics. She also had several moderate to severe reactions on blood transfusions. LE-cell tests were made on 3 occasions but were negative. On *post mortem*, there were signs of previous pericarditis and pleuritis, healed myocarditis and endocarditis, and slight chronic nephritis. The diagnostic manifestations of SLE were absent, also on re-investigation in 1963 (Prof C-G Ahlström).

Urticaria was a common phenomenon even in the absence of drug therapy Recurrent urticaria was a prominent feature of the patient s history in a few cases:

Proband 18 (group A), female born 1933, was subjectively healthy until July *1958* when she developed frequently recurrent hives. In October 1958 she had also arthralgia. In February *1959* widespread urticaria was noted, as were Raynaud' phenomenon, cervical lymphadenitis, slight leucopenia, ESR 5—43 mm, and hypergammaglobulinemia (2.5 g per 100 ml). The LE-cell phenomenon was, however not demonstrable Temporary improvement of urticaria on corticosteroids, soon followed by new spells. In the summer of *1960* nearly daily spells of hives, and also some episodes f abdominal pain and vomiting. Besides urticaria, general enlargement of peripheral lymph nodes were noticed, and the patient had ESR 22—38 mm/1 hour gammaglobulin 1.8 g per 100 ml, thrombocyte counts 120—140,000 positive SSC-test (512) but no other laboratory abnormalities. On the day of subcutaneous testing with anticatarrh vaccine the patient had joint and tendon sheath swelling, and edema of the upper lip. Intracutaneous testing with 38 different antigens was however performed with negative result. The patient was put on elimination diet and was temporarily improved. In October 1960 another attack of hives in connection with penicillin treatment, but new spells occurred without obvious connection with drugs or food. In December 1960 the patient had an acut course of SLE with fever pericarditis, bilateral pleurisy myocarditis, and nephritis with considerable proteinuria. In 1959 and 1960 LE-cell test had been performed on 6 various occasions ith negative results, but in December 1960 positive preparation was obtained. The patient died in March *1961* in general cachexia. *Post mortem* showed mitral stenosis, polyserositis, "onion-skin lesions in the spleen and "wire-loop lesions in the kidneys.

Eight of the patients reacted unfavourably to *blood transfusion* and the reaction varied in severity from chills with only slight nausea to severe shock (proband 9 page 55).

All types of hypersensitivity occurred in both probands of group A and probands of group B. They often occurred before the diagnosis of SLE had been made—sometimes months to years before the appearence of manifestations enabling the diagnosis.

The high frequency of allergic reactions in the present probands agrees with that reported by other authors. According to Harvey *et al* (1954) the reactions are not common *before* the diagnosis of SLE which was interpreted as if the development of SLE gave rise to a state of altered reactivity which was then associated with the frequent occurrence of allergic phenomena. Judging from the present probands' medical histories, the frequency of allergy was roughly equal before and after the diagnosis. This elucidates the diagnostic problem, namely: When in a series of events shall SLE be regarded as having started?

Apart from the high incidence of hypersensitivity reactions the SLE process sometimes became acute or radically worse following treatment with certain drugs (see Leonhardt 1957).

AUTOIMMUNITY

Many of the manifestations described above (LE-cell phenomenon, false-positive STS, hemocytolytic conditions *etc*) are examples of autoimmune processes typical of SLE. Of other autoimmune serologic phenomena noted in the present proband series, mention should be made of circulating anticoagulants, which were demonstrated in 3 patients (all of group A).

GENETIC FACTORS

These will be analysed in the following chapters.

GENEALOGIC STUDIES IN PROBANDS

COUSIN MARRIAGES

Of the 57 pedigrees of group A probands, 32 (56.1 %) could be completed sufficiently to judge the incidence of marriages between cousins among the parents of the probands. Only 1 such instance was found, and the *minimum* figure for cousin marriages was thus 3.1 %. However no definite conclusions about the significance of consanguinity in the genetics of SLE can be drawn from this single instance.

Of the 52 pedigrees of group B probands, 27 (51.9 %) were completed and no marriages between cousins were found. The reason why 25 pedigrees of group A and 25 of group B probands were not complete was partly because one of the parents or members of previous generations were unknown and partly because some of the parish registers were incomplete.

The parents of probands KG were known, through previous work (Larsson and Leonhardt 1961), not to be cousins.

RELATIONSHIPS BETWEEN PEDIGREES

The following relationships between pedigrees were found in the present series: (1) *Proband 5* had a distant female relative (proband 93) with suspected SLE who was included in this investigation. This relationship was reported in a previous publication (Larsson and Leonhardt 1961). (2) *Proband 10* (definite SLE) and *proband 47* (also definite SLE) were found to be *first cousins* (see Figure 6, page 115) (3) *Proband 94* (suspected SLE) had a niece with suspected SLE, who was included in the present series as *proband 104* (see Figure 9 page 119)

These observations of relationships between SLE pedigrees strengthen the hypothesis that hereditary factors play a role in the causation of SLE.

INTERVIEW OF RELATIVES AND CONTROLS

In this chapter certain data obtained from the respondents by personal interview or by questionnaire per post will be described. Concerning data suggesting collagen or autoimmune disease additional information was obtained by study of hospital records. A more detailed account of SLE like syndromes in the respondents, as judged by the cumulative information by interview *etc* and by laboratory investigation, will be given in Chapter 13.

INTERVIEW OF RELATIVES AND CONTROLS A+B

COMPLETENESS OF STUDY

A survey is given in Table 21 of *relatives A* and in Table 22 of *relatives B*. Of a total of 231 relatives A living in Scania at time of study 225 (97.4 %) were examined by interview and blood specimen. The same completion rat (96.0 %) was obtained for relatives B 217 out of 226 responded.

Probands relatives living outside Scania at the time of the study were examined only by questionnaire per post. Some of these could not be traced and some did not return the questionnaires. The completion rate was 60.4 % for probands A and 51.6 % for probands B living outside Scania.

A survey of the *controls* is given in Table 23. Of a total of 355 spouses living in Scania at the time of the study 324 (91.3 %) consented to examination by interview and blood specimen. Controls living outside Scania were not studied and no attempt was made t get information about deceased controls.

Table 21 Survey of relatives of group A probands

Relatives	Living in Scania		Living outside Scania		Dead	Unknown
	Total	Examined[b]	Total	Examined[c]		
F thers	17	17	1	1	36	3
Mothers	23	23	0	0	33	1
Brothers	64	63	22	10	13	4
Sisters	55	54	19	16	8	4
Sons	37	35	1	0	3	0
Daughters	33	31	5	2	0	0
Total male relatives	118	115	24	11	52	7
Total female relatives	113	110	24	18	41	5

Aged 10 years or more By personal interview and blood specimen By questionnaire per post

Table 22. Survey of relatives of group B probands

Relatives[1]	Living in Scania		Living outside Scania		Dead	Unknown
	Total	Examined[2]	Total	Examined[3]		
Fathers	11	11	0	0	36	5
Mothers	16	16	0	0	35	1
Brothers	65	58	16	7	24	0
Sisters	52	52	14	7	19	1
Sons	44	42	0	0	2	0
Daughters	38	38	3	4	0	0
Total male relatives	120	111	13	6	62	5
Total female relatives	106	106	18	10	54	2

[1] Aged 10 years or more [2] By personal interview and blood specimen [3] By questionnaire per post

The completion rate of 96—97 % for probands relatives in entering the examination by interview and blood specimen can be regarded as very good, the corresponding completion rate of 91 % for controls as satisfactory. Attempts were made to obtain information about the subjective health of the 15 relatives (6 of group A and 9 of group B) and the 31 controls not responding. None was said to have any gross collagen or autoimmune disease.

SEX AND AGE OF RESPONDENTS LIVING IN STUDY AREA

Table 24 shows that the control groups A and B included relatively more males than females, while the sex distribution of the groups of probands' relatives was equal. The over-representation of men in the control series is explained by the fact that this included the married partners of *probands* who were usually females. The difficulties liable to arise in the statistic comparisons owing to differences in sex

Table 23. Survey of controls. Only spouses living in Scania at time of study were used as controls. Spouses of probands and relatives A were pooled with spouses of proband KG and of her relatives to form "control group A". Spouses of probands and relatives B constitute "control group B".

Spouses of	Group A		Group KG		Group B	
	Total	Examined[1]	Total	Examined[1]	Total	Examined[1]
Female probands	30	28	1	1	30	28
Male probands	6	6	—	—	3	3
Probands' sisters	43	40	7	5	38	34
Probands' brothers	55	53	3	2	38	34
Probands' daughters	14	13	0	0	20	18
Probands' sons	14	13	0	0	25	22
Proband's KG's nieces	—	—	15	13	—	—
Proband KG's nephew	—	—	13	11	—	—
Total male spouses	87	81	23	19	88	80
Total female spouses	78	72	16	13	66	59

[1] By interview and blood specimen

Table 24. Mean ages of probands' relatives and of controls at time of study

Group	Sex	Number in group	Mean age	SD	SE
Relatives A	Males	115	43.9	16.79	1.57
	Females	110	43.5	19.93	1.90
Relatives B	Males	111	44.1	18.61	1.77
	Females	106	42.0	17.34	1.68
Controls A	Males	100	45.6	13.19	1.32
	Females	85	41.2	12.29	1.33
Controls B	Males	80	47.2	13.69	1.53
	Females	59	46.1	13.16	1.71
Controls A+B	Males	180	46.3	13.40	1.00
	Females	144	43.2	12.84	1.07

were eliminated by treating the males and females separately.

The *mean ages* of controls and of probands' relatives at time of study are given in Table 24. The mean ages of male *relatives A* did not differ from those of male *relatives B* and no difference was seen between the corresponding groups of females either.

There was no significant difference between male *controls* of group A and of group B and only a slight difference (4.9 ± 2.2) between female controls of the 2 groups.

The mean ages of the pooled controls A + B did not differ significantly from those of relatives A or of relatives B respectively—each sex considered separately.

In many of the subsequent statistic comparisons the respondents are grouped according to age mainly because genetic factors might have their maximal effect at a certain age and the effect would be masked if the respondents of this age were analysed together with the respondents of all other ages. In order not to make the analysis unnecessarily difficult, the respondents were simply divided into 2 age classes, namely below 45 years and 45 years or more.

RESIDENCE AND SOCIO-ECONOMIC STATUS OF RESPONDENTS LIVING IN STUDY AREA

The distribution of probands' relatives and of controls according to their *places of residence* is shown in Table 25. Statistic analysis gave the following results.

	χ^2	DF	P
Between relatives A and relatives B	19.47	2	<0.001
Between controls A and controls B	18.93	2	<0.001
Between relatives A and controls A + B	9.—	2	<0.01
Between relatives B and controls A + B	3.33	2	>0.10

There was thus a significant difference between the *relatives* of group A and of group B. Of relatives B a larger proportion lived in Malmö which might be expected since there were more residents of Malmö in group B than in group A *probands*.

Table 25 Residence of probands' relatives and of controls

Group[1]	Total in group	Place of residence		
		Malmö	Other towns	Rural districts
Relatives A	225	57	45	123
%		25.3	20.0	54 7
Relatives B	217	98	36	83
%		45.2	16 6	38.2
Controls A	185	51	46	88
%		27 6	24.9	47.5
Controls B	139	70	18	51
%		50.4	12.9	36.7

[1] Males and females pooled, all ages

Since the *controls* consisted of the married partners of probands and probands re latives, the same difference prevalled between the 2 groups of controls.

When relatives of group A were compared with the pooled control groups A + B, the difference was significant but the difference between relatives B and controls A + B was insignificant.

	χ^2	*DF*	*P*
Between relatives A and relatives B	7 78	2	<0.05
Between control A and controls B	4.92	2	>0.05
Between relatives A and controls A + B	0.31	2	>0.80
Between relatives B and controls A + B	2.57	2	>0.20

The probands' relatives and the controls are classified according to *socio-economic classes* in Table 26 Statistic analysis showed

A significant difference was thus found between the 2 groups *of relatives*, in that socio-economic class III was over-represent ed in relatives B No significant differen-

Table 26. Socio-economic status of probands' relatives and of controls

Group[1]	Total in group	Socio-economic class		
		I	II	III
Relatives A	225	12	79	134
%		5.3	35.1	59.6
Relatives B	217	18	51	148
%		8.3	23.5	68.2
Controls A	185	6	65	114
%		3.3	35.1	61 6
Controls B	139	12	41	86
%		8.6	29.5	61 9

Males and females pooled; all ages

Table 27 Subjective stat of health of probands' relatives

Relati es A		Relatives B	
Males under 45 years		*Males under 45 years*	
Total	62	T tal	56
No. with impaired health	1	No. with impaired health	7
Back pain, traumatic	1	Cardiovascular disease	1
		Hepatitis, chronic	1
		Mediastinitis, chronic	1
		Goitre, massive	2
		Polio, sequelae	1
		Polyarthritis, chronic	1
Males 45 years and over		*Males 45 years and over*	
Total	53	Total	65
No. with impaired health	12	No. with impaired health	11
Arthralgia, pronounced	1	Arthralgia, pronounced	1
Asthma, bronchial	1	Bronchitis, chronic	3
Bronchitis, chronic	1	Cardiovascular disease	4
Cardiovascular disease	3	Gouty arthritis	1
Cerebrovascular disease	2	?Malignant disease	2
Epilepsy	1		
Polyarthritis, chronic	2		
Prostatism	1		
Females under 45 years		*Females under 45 years*	
Total	57	T tal	60
No. with impaired health	7	No. with impaired health	8
Anemia, sideropenic	1	Arthralgia, pronounced	1
Asthma, bronchial	1	Asthma, bronchial	2
Hepatitis, chronic	1	Cardiovascular disease	1
Polyarthritis, chronic	3	Goitre massive	1
SLE	1	Polyarthritis, chronic	3
Females 45 years and over		*Females 45 years and over*	
Total	53	Total	46
No. w th impaired health	16	No. with impaired health	14
Asthma, bronchial	2	Arthralgia, pronounced	1
Cardiovascular disease	6	Cardiovascular disease	6
Cerebrovascular disease	1	Cerebrovascular disease	1
Diabetes mellitus	1	Diabetes mellitus	1
Hepatitis, chronic	1	Epilepsy	1
Polyarthritis, chronic	3	Myeloma	1
?Systemic sclerosis	1	Polyarthritis, chronic	2
		SLE, suspected	1

ces were found between the 2 groups of *controls* or between relatives A and relatives B, respectively and th pooled controls

SUBJECTIVE STATE OF HEALTH OF RESPONDENTS LIVING IN STUDY AREA

All f the respondent were q estioned regarding their present *stat of health*. All who replied that they were healthy are hereinafter referred t as subjectively healthy" the remainder as respondents with impaired health" In this classification slight physical disorders, such as common cold minor traumatic lesions, and symptoms of probably psychic nature were ignored. When a respondent had two or more co-existing diseases, nly the one mainly responsible for impairment of health is given The names of the diseases were often known by the respondents themselves, and in many cases, particu larly in those where the subjects reported symptoms suggesting collagen or autoim

Table 28. Subjective state of health of controls

Controls A		Controls B	
Males under 45 years		*Males under 45 years*	
Total	49	Total	36
No. with impaired health	2	No. with impaired health	0
Asthma, bronchial	2		
Males 45 years and over		*Males 45 years and over*	
Total	51	Total	44
No. with impaired health	6	No. with impaired health	11
Back pain (herniated disc)	1	Arthralgia, pronounced	2
Bronchitis, chronic	1	Bronchitis, chronic	1
Cardiovascular disease	3	Cardiovascular disease	5
Prostatism	1	Cerebrovascular disease	1
		Multiple sclerosis	1
		Parkinsonism	1
Females under 45 years		*Females under 45 years*	
Total	55	Total	22
No. with impaired health	4	*No. with impaired health*	1
Anemia, sideropenic	2	Bronchitis, subacute	1
Arthralgia, pronounced	1		
?Malignant disease	1		
Females 45 years and over		*Females 45 years and over*	
Total	30	Total	37
No. with impaired health	6	No. with impaired health	10
Asthma, bronchial	1	Arthralgia, pronounced	1
Cardiovascular disease	1	Asthma, bronchial	2
Cerebrovascular disease	1	Cardiovascular disease	2
Epilepsy	1	Cerebrovascular disease	1
?Malignant disease	1	Diabetes mellitus	1
Polio, sequelae	1	Multiple sclerosis	1
		Polyarthritis, chronic	2

mune diseases, the diagnosis was checked in the hospital records. In the remaining cases the diagnosis was judged by the the author

The diseases reported by *the probands' relatives* are given in Table 27 Of *relatives A* under 45 years of age, 3, all women, had chronic polyarthritis. Two other patients had severe immunopathies, one had definite SLE (she had been excluded from the proband material because her sister proband 5 had already been included) 1 had chronic hepatitis (a sister of proband 54). Of the relatives A over 45 years, 6, including 2 males and 3 females with chronic polyarthritis, had arthralgia. One woman (the mother of proband 9) showed clear cut scleroderma on inspection another (the sister of the same proband) had chronic hepatitis.

Of *relatives B* under 45 years, 5 including 1 male and 3 females with chronic polyarthritis, had arthralgia. Two males and 1 female belonging to the same sibship had massive goitre, which had recurred after operation. One man had an obscure disease that had been classified as chronic mediastinitis" The groups above 45 years comprised 4 with arthralgia, including 2 females with chronic polyarthritis. One female had suspected SLE (and had not been accepted as a proband because her sister proband 95 had already been included in the proband group B). One woman had a tumour originating from the antibody forming system, namely myeloma.

The groups above 45 years, both of relatives A and of relatives B, included several cases of cardiovascular disease hemiplegia, arteriosclerotic demens, suspected malignant tumours *etc*.

Table 29 Incidences of collagen diseases and of other diseases, as judged from information obtained at interview and from hospital records, in probands' relatives and in controls living in Scania at time of study

Sex	Age (years)	Relatives A			Relatives B			Controls A+B		
		Total	Number with		Total	Number with		Total	Number with	
			Collagen diseases	Other diseases		Collagen diseases	Other diseases		Collagen diseases	Other diseases
Males	<45	62	0	1	56	2	5	85	0	2
	≥45	53	2	10	55	0	11	95	0	17
	T tal	115	2	11	111	2	16	180	0	19
	%		1.7	9.6		1.8	14.4		0.0	10.6
Females	<45	57	5	2	60	3	5	77	0	6
	≥45	53	5	10	46	3	11	67	2	14
	Total	110	10	12	106	6	16	144	2	19
	%		9.1	10.9		5.7	15.1		1.4	13.2

Only few of the *controls* below 45 years had impaired health (Tabl 28). Only 1 control (female) complained of pronounced joint pain at time of interview In the groups above 45 years cardiovascular disease (hypertension, angina pectoris, cardiac incompensation) was the commonest cause of impaired health. One man and 1 woman were invalided by multiple sclerosis. One man had post-encephalitic parkinsonism. Only 5 persons (3 females and 2 males) complained of pronounced arthralgia and of these 2 females had chronic poly arthritis.

All the respondents (8 relatives A, 6 relatives B and 2 controls) given the diag nosis *chronic polyarthritis* in Tables 27 and 28 had persistent joint symptoms and typical symmetric swellings of the metacarphophalangeal and proximal interphalangeal joints, often with deformities, at time f interview Many of them had n some previous occasion been in hospital because of the joint disease

The 11 relatives with chronic polyarth ritis included 4 (2 group A and 2 group B) relatives of *probands with chronic polyarthritis* while the remainder were relatives of probands with acute-subacut polyarthritis (4 group A and 2 group B) or with only arthralgia (2 of each group).

Besides in the respondents labelled as chronic polyarthritis in Tables 27 and 28, joint deformities of rheumatoid type were found in additional 2 female relatives A, which were, however listed under the headings of SLE and ?systemic sclerosis, respectively

Four relatives (1 of group A and 3 of group B) and 4 controls had pronounced arthralgia at the time of the author's examination but did not show any gross signs of joint affection. It should be noted, however that th respondents were not subjected to *thorough* clinical examination. Therefore no attempt was made to assess the incidence of the different forms of rheumatoid arthritis according to the American Rheumatism Association criteria.

Table 29 compares the incidences of disease in relatives of the probands and in the controls. In this table, all subjects with chronic polyarthritis, chronic hepa titis, systemic sclerosis or SLE were grouped under the heading of "collagen diseases" Controls A and controls B showed n evident difference as t their

Tabl 30 Incidences of previous and present rheumatic complaints in probands' relatives and controls

Sex	Age (years)	Relatives A Total	Relatives A No. with complaints from: Periph joints	Prox joints	Spine	Relatives B Total	Relatives B No. with complaints from: Periph joints	Prox joints	Spine	Controls A + B Total	Controls A + B No. with complaints from: Periph joints	Prox joints	Spine
Males	<45	62	1	4	1	56	6	3	2	85	5	2	3
	≥45	53	9	6	2	55	9	9	4	95	6	12	6
	Total	115	10	10	3	111	15	12	6	180	11	14	9
	%		8.7	8.7	2.6		13.5	10.8	5 4		6.1	7.8	5.0
Females	<45	57	9	0	1	60	8	6	1	77	4	6	1
	≥45	53	14	10	5	46	14	3	6	67	14	8	7
	Total	110	23	10	6	106	22	9	7	144	18	14	8
	%		20.9	9 1	5.5		20.8	8.5	6.6		12.5	9 7	5.6

state of health and they have been pooled in the table

Statistic analysis, with adjustment for sex and age, gave as follows:

Respondents with collagen diseases	*Differ ence*	*Mean error*	*P*
Relatives A compared with relatives B	+ 1.8	2.2	>0.40
Relatives A compared with controls A + B	+ 6 1	1.8	<0.001
Relatives B compared with controls A + B	+ 4 1	1.5	<0.01

Respondents with other diseases	*Differ nce*	*Mean error*	*P*
Relatives A compared with relatives B	— 5 1	3 4	>0.10
Relatives A compared with controls A + B	— 1.5	3.5	>0.60
Relatives B compared with controls A + B	+ 4.4	3.7	>0.30

Thus, collagen diseases were more com mon among the relatives (A as well as B) than among controls, while other diseases were equally common in both categories.

RHEUMATIC COMPLAINTS OF RESPONDENTS LIVING IN STUDY AREA

The respondents were questioned regard ing previous and present *joint pain* and *swelling* of the joints. They were also asked which joints were *involved most*. Traumatic joint affection was ignored.

The results of this inquiry are given in Table 30 where previous and present rheumatic complaints have been pooled. Respondents with complaints mainly of the joints of the hands and feet were said to have affection of peripheral joints those with symptoms mainly from the joints of the elbows, shoulders, hips or knees, to have affection of proximal joints and those with back pain to have affection of spine. From the last category however *respondents with symptoms suggesting* herniated disc syndromes have been ex cluded.

The table shows that affection of the peripheral joints was more common among female than among male *controls* while affection of proximal joints and of the spine was equally common in both sexes. Present or previous rheumatic complaints of some kind were reported in 18.9 % of male controls and in 27.8 % of female controls.

Table 30 shows no noteworthy differ ences between *relatives A* and *relatives B* concerning the frequency of the different types of rheumatic complaints. Distinct

differences between relatives A or relatives B on one hand and controls on the other were found only for one type of complaints, namely affection of peripheral joints:

Respondents with symptoms from peripheral joints		*Difference*	*Mean error*	*P*
Relatives A compared with relatives B	—	2.8	3.7	>0.40
Relatives A compared with controls A + B	+	7.2	3.6	<0.05
Relatives B compared with controls A + B	+	10.5	3.7	<0.01

OTHER PHYSICAL ABNORMALITIES OF RESPONDENTS LIVING IN STUDY AREA

Tables 31 and 32 give the incidences of present and previous physical abnormal ities relevant to SLE and reported by the respondents.

Regarding certain abnormalities, the respondents gave such vague information that they are not accounted for here. This applies, for example to anemia—several women reported that they had had anemia, but in most cases this was probably simpl iron deficiency anemia. Affec tion of the yes and dryness of the mouth (Sjögren s syndrom) proved very difficult t j dge and were therefore ignored.

Regarding the incidences of the physical abnormalities included in T ble 31 no differences were found between relatives A and relatives B. Neither were any systematic differences found between the relatives of the probands and the controls, except regarding "inflammation of the lungs which was strikingly high among the female relatives f group A probands, but this difference might be irrelevant.

Several controls as well as relatives of probands stated that they had had epidemic *hepatitis* which had however been of short duration and had not left any sequ lae *Chroni hepatitis* on the other hand, was unknown i th controls but present in 3 of the relatives (1 of group A and 2 of group B) furthermore another female relative of a group B proband (number 95) had chronic hepatitis combined with Sjögren s syndrome possibly classi fiable as "suspected" SLE. These cases are reported in some detail later in Chapter 13.

Thyroid disease did not seem to be more common in the relatives of probands than in the controls. Most cases of thyroid disease consisted of toxic goitre, which had usually been operated upon. Only 3 re spondents reported that they had thyroid hypofunction that necessitated substitu tion therapy In 5 respondents the thyroid was enlarged without producing endocrine symptoms. Judging from the information available (the personal reports from the respondents *and* the hospital records from those respondents who had been in hospital because of thyroid disease) none of the controls or relatives had had frank thyroiditis of Hashimoto s type.

Table 32 gives the incidences of *sensi tivity to cold* and of previous and present *allergies* Sensitivity to cold was said to be present when the respondents reported spells of pallor and numbness of the hands and feet when exposed to cold. A few re spondents also had *cyanosis* on such occa sions, thus Raynaud s phenomenon.

There was a higher incidence of sensitivity to cold in the series of relatives than in the controls (difference between observed and expected for relatives A in comparison with controls + 7 6 mean error 2.6, cor responding figures for relatives B + 5.8 and 2.6, respectively). A tendency to higher incidences in relatives than in controls was also found for *sensiti ity t sun* and *sensitivity to drugs* although the numbers were too small to give statistically significant differences.

As to sensitivity to sunshine none of the respondents had cutaneous LE verified by clinical examination. Some relatives of the probands, however had a history suggestive of LF and t time of interview 3 relatives had erythema of the face con sistent with this diagnosis. One female control had had recurrent sunshin ex anthema diagnosed by a dermatologist as polymorph us light erythema Of the

Table 31 Incidences of some other previous and present physical abnormalities in probands relatives and in controls

Sex	Age (years)	Relatives A Total	Relatives A Number with: Pneumonia	Pleuritis	Pulm tbc	Thyroid disease	Hepatitis	Proteinuria	Relatives B Total	Relatives B Number with: Pneumonia	Pleuritis	Pulm tbc	Thyroid disease	Hepatitis	Proteinuria	Controls A+B Total	Controls A+B Number with: Pneumonia	Pleuritis	Pulm tbc	Thyroid disease	Hepatitis	Proteinuria
Males	<45	62	1	2	2	0	1	2	56	6	2	1	2	2	0	85	11	1	1	0	3	0
	≥45	53	13	0	1	1	2	0	55	12	2	1	0	2	0	95	16	4	3	0	5	3
	Total	115	17	2	3	1	3	2	111	18	4	2	2	4	0	180	27	5	4	0	8	3
	%		14.8	1.7	2.6	0.9	2.6	1.7		16.2	3.6	1.8	1.8	4.6	0.0		15.0	2.8	2.2	0.0	4.4	1.7
Females	<45	57	7	0	1	1	4	7	60	5	2	1	1	2	5	77	9	0	0	3	2	5
	≥45	53	15	0	2	5	5	4	46	8	1	1	8	1	5	67	8	0	3	6	3	6
	Total	110	22	0	3	6	9	11	106	13	3	2	9	3	10	144	17	0	3	9	5	11
	%		20.0	0.0	2.7	5.5	8.2	10.0		12.3	2.8	1.9	8.5	2.8	9.4		11.8	0.0	2.1	6.3	3.5	7.6

Table 32. Incidences of previous and present sensitivity to cold and various allergies in probands relatives and in controls

Sex	Age (years)	Relatives A Total	Sens to cold	Resp tract allergy	Urticaria (food)	Eczema	Sens to sun	Sens to drugs	Relatives B Total	Sens to cold	Resp tract allergy	Urticaria (food)	Eczema	Sens to sun	Sens to drugs	Controls A + B Total	Sens to cold	Resp tract allergy	Urticaria (food)	Eczema	Sens to sun	Sens to drugs
			Number with							Number with							Number with					
Males	< 15	62	2	4	1	1	0	0	56	3	6	4	4	0	1	85	0	4	1	4	0	0
	≥ 15	53	0	8	1	2	1	3	55	6	5	4	5	0	1	95	2	4	4	10	0	0
	Total	115	2	12	8	3	1	3	111	9	11	8	9	0	2	180	2	8	5	11	0	0
	%		1.7	10.4	7.0	2.6	0.9	2.6		8.1	9.9	7.2	8.1	0.0	1.8		1.1	4.4	2.8	7.8	0.0	0.0
Females	< 15	57	10	3	7	5	6	3	60	9	5	5	8	2	0	77	3	3	4	10	3	0
	≥ 15	51	8	5	3	6	4	1	46	3	4	3	5	5	1	67	4	12	8	6	1	2
	Total	110	18	8	10	11	10	7	106	12	9	8	13	7	1	144	7	15	12	16	4	2
	%		16.4	7.3	9.1	10.0	9.1	6.4		11.3	8.5	7.5	12.3	6.6	0.9		4.9	10.4	8.3	11.1	2.8	1.4

Table 33. Survey of relatives of proband KG

Relatives	Living in Scania		Living outside Scania		Dead
	Total	Examined[a]	Total	Examined[a]	
Father	—	—	—	—	1
Mother	—	—	—	—	1
Brothers	4	3	0	0	0
Sisters	7	7	1	1	1
Nephews	22	19	0	0	0
Nieces	24	18	2	1	1
Total male relatives	26	22	0	0	1
Total female relatives	31	25	3	2	3

Aged 10 years or more By interview and blood specimen

respondents who reported drug reactions 2 (relatives of the probands) stated that they were hypersensitive to *penicillin.* Of other hypersensitivity reactions, mention might be made of 1 case of *agranulocytosis following sulfa therapy* in a male relative of group A (brother of proband 18)

RESULTS OF INTERVIEW OF RELATIVES LIVING OUTSIDE STUDY AREA

Of the 29 *relatives A* living outside Scania at the time of study and answering questionnaires sent per post, 8 had previous or present rheumatic complaints—4 peripheral and 4 proximal. Of these, 2 stated that they had chronic polyarthritis and the diagnosis was confirmed by study of hospital records in 1 No other diseases of special interest in connection with SLE were reported by these relatives.

Sixteen *relatives B* living outside study area returned the questionnaires. Six reported previous or present rheumatic complaints: 2 from peripheral, 2 from proximal joints and 2 complaints from the spine. None appeared to suffer from chronic polyarthritis, and no other diseases of interest were reported.

INTERVIEW OF RELATIVES KG

COMPLETENESS OF STUDY

A survey of the relatives of proband KG is given in Table 33. Of a total of 60 relatives KG (all but 3 living in study area) 49 (81 7 %) were examined by interview and blood specimen.

SUBJECTIVE STATE OF HEALTH

Of the relatives of the proband KG only 4 all sisters of the proband stated that their *health was impaired* at the time of interview One of these was the ubject of a previous publication (Leonhardt 1957) and reported to have SLE. Another previously healthy sister recently developed cutaneous changes consistent with LE. A third sister had shortly before the inter view been in hospital because of tuber culous *lymphadenitis* and suspected *tuber culous pleuritis;* she had also had a tran sient *face rash* diagnosed as discoid LE by a dermatologist. Finally another sister complained of cardi vascular symptoms. No cases of chronic polyarthritis were found in the relatives KG studied.

RHEUMATIC COMPLAINTS

Previous or present rheumatic complaints were reported by 1 brother (proximal arthralgia), 4 sisters (2 peripheral and 2 proximal arthralgia), 1 nephew (proximal arthralgia) and 3 nieces (all peripheral arthralgia) of the proband. Thus 9 (18.4 %) out of 49 relatives KG answered in the affirmative t the question concerning rheumatic complaints; the incidence is not higher than that found in the controls (= 22.8 % in controls A + B males and females combined, all ages).

OTHER PHYSICAL ABNORMALITIES

Concerning other physical abnormalities the only remarkable finding was the incidence of *sensitivity to sun* which was reported by 5 relatives (10.2 %). Four of these were sisters of the proband KG, and 3 had cutaneous LE verified by dermatologic consultants. One niece was probably hypersensitive to penicillin; she had previously had a symptom complex labelled as Henoch Schönlein's syndrome but reminding of SLE (Larsson and Leonhardt 1959).

COMPARISON WITH PUBLISHED SERIES

For comparison with published series regarding physical abnormalities in SLE relatives, see Chapter 13.

ANALYSIS OF BLOOD GROUPS

METHOD

The ABO- and Rh-blood types of 59 of the probands were obtained from the hospital records. The blood grouping had been done in different blood banks. No information was available concerning the blood types of 7 of the probands who had died before the time of the present study. Blood from the remaining 43 probands, all living at the time of study were grouped at the Blood Bank, Malmö General Hospital. Relatives of probands and controls were grouped according to the ABO system by the author personally. The blood cells as well as the serum were tested against known blood cells and known serum available at the Blood Bank Malmö General Hospital.

In association with a campaign in 1952 for recruitment of blood donors in Scania, the ABO and Rh blood types were determined in 5668 volunteers (Willert and Winblad 1953). The distribution in this material may be regarded as representative of the population of Scania and will be used as reference in some comparisons.

BLOOD GROUPS IN PROBANDS

The distribution of the *ABO* blood types of the *probands* is given in Table 34. Statistic analysis showed as follows.

	χ^2	DF	P
Between probands A and probands B	0.48	3	>0.90
Between probands A and controls A + B	2.39	3	>0.30
Between probands B and controls A + B	1.01	3	>0.70
Between probands A and population sample	4.54	3	>0.20
Between probands B and population sample	2.90	3	>0.30

Thus no difference in distribution of blood types was found between the two groups of probands or between probands and controls. Comparison of the blood type distribution of the probands with that in Willert and Winblad's series will give somewhat larger but not significant, χ^2-values. On examination of Table 34 it will be seen that proband group A contains numerically more individuals belonging to blood type A and fewer belonging to blood type O than does the population sample. The differences are not significant, but some relationship between definite SLE and blood group A cannot be excluded. Decision of this point requires a larger series.

As to the *Rh* blood types, 45 (83.3 %) of the 54 probands A tested were Rh-positive while 44 (91.7 %) of 48 probands B were positive. The difference was not significant ($\chi^2 = 0.87$ DF $= 1$ $P > 0.30$). In the investigation of Willert and Winblad, 83.1 % of 5668 volunteer blood donors were found to be Rh-positive. The percentages in the groups of probands were of the same order

Table 34. Distribution of ABO blood groups in probands, probands relatives, controls and population sample

Group[1]	Total tested	Blood groups A No.	A %	B No.	B %	0 No.	0 %	AB No.	AB %
Probands A (57)	54	29	53.7	4	7.4	17	31.5	4	7.4
Probands B (52)	48	23	47.9	5	10.4	16	33.3	4	8.4
Relatives A (225)	221	108	48.9	14	6.3	90	40.7	9	4.1
Relatives B (217)	213	108	50.7	21	9.9	74	34.7	10	4.7
Controls A (185)	181	69	38.1	22	12.2	80	44.2	10	5.5
Controls B (139)	138	75	54.3	16	11.6	40	29.0	7	5.1
Controls A + B (324)	319	144	45.2	38	11.9	120	37.6	17	5.3
Population sample[2]	5668	2430	42.8	590	10.4	2401	42.3	247	4.5

[1] Males and females pooled, all ages [2] Blood donor volunteers. From an investigation of Willert and Winblad (1953), by permission

BLOOD GROUPS IN RELATIVES AND CONTROLS A+B

The ABO blood type distribution of the *probands' relatives* and of the *controls* is also given in Table 34. Statistic analysis showed.

	χ^2	*DF*	*P*
Between relatives A and relatives B	2.87	3	>0.30
Between controls A and controls B	9.44	3	<0.05
Between relatives A and controls A + B	5.36	3	>0.10
Between relatives B and controls A + B	1.71	3	>0.50
Between relatives A and population sample	5.35	3	>0.10
Between relatives B and population sample	5.83	3	>0.10
Between controls A + B and population sample	3.26	3	>0.30

No difference in blood type distribution was found between the 2 groups of relatives, but a significant difference was found between the corresponding groups of controls—the control group B having a higher incidence of blood type A and a lower incidence of blood type O than controls A. When the 2 groups of controls were pooled, however the blood type distribution was not significantly different from that of the population sample.

Relatives A comprised numerically more persons of blood type A in comparison with the pooled control material and in comparison with the population sample (confer the corresponding tendency among probands A) but the differences were not statistically significant.

BLOOD GROUPS IN RELATIVES KG

The distribution among blood types of relatives KG was reported previously (Leonhardt 1959). Nothing remarkable was found in this pedigree.

COMPARISON WITH PUBLISHED SERIES

Perusal of the literature failed to reveal any reports of investigations of the blood group distributions in patients with *SLE*. No investigations of blood groups in *SLE relatives* seem to have been published.

ELECTROPHORETIC ANALYSIS OF SERUM PROTEINS

METHOD

The electrophoretic examination of sera was performed in the author's laboratory in the Royal Dental School at Malmö by one specially trained technician.

Total protein was determined by a modified Biuret technique (Reinhold 1953) *Fractionation* was done by electrophoresis on paper using Laurell s modification (Laurell *et al* 1956)

The electrophoretic examinations were carried out successively during the years 1961—1963. The *reproducibility* of the results was checked by repeated examination of one standard serum, distributed among sealed glass ampoules and kept deep-frozen.

The accuracy of the electrophoretic method employed has been discussed pre viously (Larsson and Leonhardt 1959 Belfrage 1963). The method had been found to be practical in the study of the quantitative aspects of serum proteins in different groups of individuals.

Electrophoresis of the serum proteins with this method and this set of appara tuses gave on the average higher values for albumin and lower values for the globulin fractions than those usually found in the literature and also than those obtained by electrophoresis performed at the Department of Clinical Chemistry Malmö General Hospital, and referred to in previous publications (Leonhardt 1957 Larsson and Leonhardt 1959) The nu meric values found for the electrophoretic analysis in this paper are therefore not strictly comparable with those in previously published series.

AGE AND SEX-DEPENDENCE OF GAMMAGLOBULIN

As in other large materials of electrophoretic studies of the serum proteins (Tizzani 1955), the gammaglobulin levels of probands relatives and of controls tended to rise with age and to be somewhat higher in females than in males. It was considered advantageous to adjust for age and sex before comparisons between groups of respondents. The following procedure was used.

The influence of age was estimated from the regression coefficients of the gamma globulin on 10-year age classes in the controls. The variation with age was of the same order for all groups of controls (controls A and controls B males and females) and the regression coefficient for the pooled material was 0.03 g per 100 ml per 10 year interval (Table 35) The indivi dual gammaglobulin values were adjusted to hold for an arbitrarily chosen age class, 40—49 years. This correction was done by decreasing all values in the age class 50— 9 years by 0 03 g per 100 ml, those in the 60—69 year class, by 0 06 g per 100 ml, and those in the ≥ 70 year class by 0.09 g per 100 ml. In the age classes below the 40—49 year interval the corresponding values were added instead of subtracted.

The mean gammaglobulin values, cor rected for age, of the various groups of controls are given in Table 36, in which the corresponding figures for probands relatives are included for comparison. No significant differences were found between male controls A and male controls B, or

Table 35. Gammaglobulin values of controls in relation to age

Sex	Group	Total tested	Mean age	Mean gammaglobulin value	Coefficient of regression
Males	Controls A	100	45.6	0.88	0.05
	Controls B	80	47.2	0.87	0.04
Females	Controls A	85	41.2	0.93	0.01
	Controls B	59	46.1	0.97	0.03
	Total	324	44.9	0.91	0.03

Gammaglobulin on 10-year age classes

between female controls A and female controls B. A significant sex difference was established on comparison between the pooled males (A + B) and the pooled females (A + B).

The age-corrected gammaglobulin values were then divided into standardized intervals calculated from the mean and the standard deviation of each sex. The division into 4 classes was found suitable for later comparisons between the controls and probands and probands' relatives, respectively. The classification intervals were chosen in the light of the fact that the high values were of greatest interest. The 4 standardized intervals were:

Below Mean	= 0
Mean to Mean + 1 SD	= + 1
Mean + 1 SD to Mean + 2 SD	= + 2
Mean + 2 SD and above	$\geq$ + 3

If distributed according to the normal curve 50.0 %, 34.1 %, 13.6 % and 2.3 % of a population of gammaglobulin values should fall within the standardized intervals in the order given.

Table 36. Means of the age-corrected gammaglobulin values of probands' relatives and of controls

Group	Sex	Total tested	Mean	SD	SE
Relatives A	Males	115	0.92	0.19	0.02
	Females	110	1.05	0.34	0.03
Relatives B	Males	111	0.96	0.28	0.02
	Females	106	1.09	0.47	0.05
Controls A	Males	100	0.88	0.17	0.02
	Females	85	0.91	0.20	0.02
Controls B	Males	80	0.87	0.16	0.02
	Females	59	0.96	0.21	0.03
Controls A + B	Males	180	0.87	0.17	0.01
	Females	144	0.95	0.21	0.02

Table 37 Distribution, among standardized intervals, of th age-corrected gammaglobulin values in probands relatives and in controls

Group	Sex	Total tested	Standardized gammaglobulin intervals			
			0	+1	+2	≥+3
Relatives A	Males	115	45	43	19	8
	Females	110	44	39	17	10
	Total	225	89	82	36	18
	%		39 6	36.4	16.0	8.0
Relatives B	Males	111	38	50	11	12
	Females	106	40	40	17	9
	Total	217	78	90	28	21
	%		35.9	41.5	12.9	9.7
Controls A	Males	100	52	31	14	3
	Females	85	49	22	10	4
	T tal	185	101	53	24	7
	%		54.6	28.6	13.0	3.8
Controls B	Males	80	43	29	6	2
	Females	59	29	17	12	1
	Total	139	72	46	18	3
	%		51.8	33.1	12.9	2.2

The distribution among standardized intervals of the age-corrected gammaglobu lin values of the various control groups is given in Table 37 Analysis gave as follows

	χ^2	DF	P
Between male and female controls A	1.22	3	>0.70
Between male and female controls B	3.97	3	>0.20
Between total controls A and total controls B	1.31	3	>0 70

The distribution among the standardized intervals was thus the same for the males and females and no difference was found between controls A and controls B. The distribution of the pooled control material (males + females, A + B) among the 4 intervals shows that the normal curve is applicable (Table 38). No significant difference was found between the observed and the expected distributions (χ = 2.98 DF = 3 P > 0.30)

Table 38. Distribution of the age-corrected gammaglobulin values in probands relatives and in controls, compared with expected normal distribution

Group[1]	Total tested		Standardized gammaglobulin intervals			
			0	+1	+2	≥+3
Relatives A	225	Obs	89	82	36	18
		Exp	*112.5*	*76 7*	*30 6*	*5.2*
Relatives B	217	Obs	78	90	28	21
		Exp	*108 5*	*74 0*	*29 5*	*5 0*
Controls A + B	324	Obs	173	99	42	10
		Exp	*162 0*	*110 5*	*44 1*	*7 4*

Males and females pooled

Table 39. Distribution of the age-corrected gammaglobulin values in probands

Group[1]	Total tested	Standardized gammaglobulin intervals			
		0	+1	+2	≥+3
Probands A	37	5	5	4	23
%		13.5	13.5	10.8	62.2
Probands B	35	3	5	6	21
%		8.6	14.3	17.1	60.0

[1] Males and females pooled

GAMMAGLOBULIN IN PROBANDS

All *probands* living at the time of the study were examined electrophoretically regarding their serum protein patterns. The gammaglobulin values were corrected for age with the factor 0.03 g per 100 ml per 10-years calculated for the controls and distributed among standardized intervals based on the means and standard deviations *of the controls*. The results are given in Table 39. As expected, the distribution deviated markedly from that of the controls and from the normal curve. Twenty-three (62.2 %) of group A and 21 (60.0 %) of group B probands had gammaglobulin values falling within the + 3 interval or in higher intervals (Figures 3 and 4). Some of the gammaglobulin values, however fell in the + 1 interval and some even in the 0 interval, i.e. below the mean of the controls. These low values can be explained by the fact that the blood samples were usually collected from the probands during periods of clinical remission when the patients were as a rule, also receiving corticosteroid therapy. In addition, relatively low gammaglobulin values were noted in probands with nephropathy particularly in patients with the nephrotic syndrome.

There was no difference between group A and group B probands in th distribution of gammaglobulin values among standardized intervals (Table 39).

GAMMAGLOBULIN IN RELATIVES AND CONTROLS A+B

Fo comparison with the control material the individual gammaglobulin values in the *probands' relatives* were corrected for age and distributed among standardized i tervals in the same way as the values of th probands. The distributions thus obtained are given in Table 37 and in Figures 3 and 4. Statistic analysis of the distributions in Table 37 gave:

	χ^2	DF	P
Bet een male and female relatives A	0.18	3	>0.93
Bet een male and female relatives B	2.76	3	>0.50
Bet een pooled male and female relatives A and pooled male and female relatives B	2.82	3	>0.30

The distribution among the standardized intervals was thus th same for males and f males in relatives A as well as in relatives B. Neither did the distribution of all values within relatives A differ from that of ll val es within relatives B. These distributions, however differed significantly from that of a normal curv (χ^2 = 31.05 for relatives A and 64.16 for relatives B) (Table 35).

STANDARDIZED INTERVALS

SUBJECTIVELY HEALTHY
x HEALTH IMPAIRED

-3 | -2 | -1 | 1 | +2 | +3 | +4 | +5 | +6 | +7 | +8 | +9 | +10

PROBANDS A

PROBANDS B

RELATIVES A

RELATIVES B

CONTROLS A

CONTROLS B

GAMMAGLOB (in g per 100 ml) 0.50 1.00 1.50 2.00 2.50

Figure 3. Distribution of age-corrected gammaglobulin values in male probands, probands relatives and controls

STANDARDIZED INTERVALS

3 | -2 | -1 | +1 | +2 | +3 | +4 | +5 | +6 | +7 | +8 | +9 | +10

PROBANDS A

PROBANDS B

RELATIVES A

RELATIVES B

CONTROLS A

CONTROLS B

GAMMAGLOB (in g per 100 ml) 0.50 1.00 1.50 2.00 2.50 3.00

Figure 4. Distribution of age-corrected gammaglobulin values in female probands, probands relatives and controls

Table 40. Subdivision in hypothetic "normal" and hypothetic "hypergammaglobulinemic" population of the observed gammaglobulin values (age-corrected and distributed in standardized intervals) of probands' relatives

Group[1]	Population of gammaglobulin values	Total in population	Standardized gammaglobulin intervals			
			0	+1	+2	≥+3
Relatives A	Observed	225	89	82	36	18
	"Normal"	178	89	61	24	4
	Difference: "Hypergammaglobulinemic"	4	—	21	12	14
Relatives B	Observed	21	78	90	28	21
	"Normal"	156	78	53	21	4
	Difference: "Hypergammaglobulinemic"	61	—	37	7	1

[1] Males and females pooled

When the gammaglobulin distributions of the probands' relatives and of the controls were compared (Table 37) the following differences became evident.

	χ^2	DF	P
Between relatives A and controls A + B	13.24	3	<0.01
Between relatives B and controls A + B	22.62	3	<0.001

Thus a significant difference was found between relatives A and controls and significant between relatives B and controls, the high gammaglobulin values being clearly overrepresented in the groups of relatives. The differences between the relatives and the controls were still more obvious when the gammaglobulin values falling over the + 2 interval were subdivided further in standardized intervals of higher dignity. Several gammaglobulin values in the relatives were found in the intervals between + 3 and + 10 or even higher. Only a few controls had values falling within the + 3 and the + 4 intervals, and none had still higher values (Figures 3 and 4).

While the values noted in the controls, as described previously fitted a normal distribution, those noted in the relatives did not (Table 38). One might imagine the existence of 2 populations of gammaglobulin values, one corresponding to the distribution of the controls—roughly a normal one— the other showing a higher mean value and a wider scattering. Assuming the latter "hypergammaglobulinemic" population not to include values below the mean of the controls, the division would be that shown in Table 40.

GAMMAGLOBULIN AND SUBJECTIVE STATE OF HEALTH

Table 41 gives the distribution among standardized intervals, of the age-corrected gammaglobulin values in subjectively healthy probands' relatives and controls and those with impaired health. In the latter the gammaglobulin levels clearly tended to be higher. After pooling of relatives and controls, the difference between healthy and sick turned out to be significant (χ^2 = 36.49, DF = 3, P < 0.001). It should be remembered that the respondents (especially the relatives) included several cases of gross collagen diseases, in which hypergammaglobulinemia is a characteristic feature.

The differences between relatives and controls regarding the distribution of the gammaglobulin values persisted even after

Table 41. Distribution of the age-corrected gammaglobulin values in probands' relatives and in controls, divided according to their subjective state of health

Group	State of health	Total tested	Standardized gammaglobulin intervals			
			0	+1	+2	≥+3
Relatives A	Healthy	190	77	73	29	11
	Health impaired	35	12	9	7	7
Relatives B	Healthy	177	68	78	20	11
	Health impaired	40	10	12	8	10
Controls A+B	Healthy	284	156	85	38	5
	Health impaired	40	17	14	4	5
Total relatives	Healthy	651	301	236	87	27
	%		46.2	36.3	13.4	4.1
and controls	Health impaired	115	39	35	20	21
	%		33.9	30.4	17.4	18.3

Males and females pooled

exclusion of the respondents with impaired health.

	χ^2	DF	P
Between healthy relatives A and healthy relatives B	1.92	3	>0.50
Between healthy controls A and healthy controls B	1.05	3	>0.70
Between healthy relatives A and healthy controls A + B	15.11	3	<0.01
Between healthy relatives B and healthy controls A + B	18.89	3	<0.001

HYPERGAMMAGLOBULINEMIA"

It was stated above (page 79) that of a normal distribution of gammaglobulin values, 13.6 % would fall within the + 2 standardized interval and 2.3 % in the higher intervals. Roughly these figures were found for the controls (Table 37). It sh uld then be reasonable to regard those (age-corrected) gammaglobulin values fall ing within the + 2 standardized interval as indicating "slight hypergammaglobulin emia and those falling in the higher in tervals as indicating "hypergammaglo-bulinemia Gammaglobulin values in the + 5 or higher intervals were never seen in the controls and were said to indicate "pronounced hypergammaglobulinemia"

Only 2 male relatives (both of group B) had *pronounced hypergammaglobulinemia*. Of these the 54 year-old brother of proband 77 was requested to come for closer investigation at the Department of Medi cine, Malmö General Hospital. The posi tive findings were peribronchitis of the left lung base on X ray and an earlier diagnosed right sided renal calculus—no signs of urinary infection. The calculus was later removed at operation. The serum proteins were re investigated 2 years after operation, and pronounced hypergamma-globulinemia (2.1 g per 100 ml—not correc ted for age) persisted. The patient had no other serum protein abnormalities. The other male relative with pronounced hyper gammaglobulinemia the 77 year-old broth er of proband 109 could not be persuaded to be investigated closer He was, however subjectively healthy A positive FII AP (160) and FIIA SC-test (128) might speak in favour of essential hypergammaglo-bulinemia (Waldenström 1944).

Ten female relatives (5 of each group A and B) showed pronounced hypergamma

Table 42. Distribution of the age-corrected gammaglobulin values in relatives KG

Group	Total tested	Standardized gammaglobulin intervals			
		0	+1	+2	≥+3
Brothers	3	0	1	1	1
Sisters	8	0	1	2	5
Nephews	19	3	8	5	3
Nieces	19	6	5	4	4
Total	49	9	15	12	13
%		18.4	30.6	24.5	26.5

globulinemia. Of these, 6 had one or another of the gross collagen diseases (SLE, chronic polyarthritis *etc*) and 1 (the 55-year-old sister of proband 105) was known to have myeloma. The remaining 3 had, on special investigation, no signs permitting a "specific" diagnosis and could properly be termed "essential" hypergammaglobulinemia. These cases will be described in Chapter 13.

Gammaglobulin of *monoclonal* appearance was present only in the female relative of group B known to have myeloma. However a narrow band was found in the beta-2 fraction in the serum of the 63-year old father of proband 39 (group A). He was subjectively healthy and had as the only other abnormalities positive SSC- (64) and borderline FIIA-SC-tests (32). He could not be investigated closer

"HYPOGAMMAGLOBULINEMIA"

It follows from Figures 3 and 4 that none of the SLE relatives or of the controls had agammaglobulinemia. Gammaglobulin values in the "low" standardized intervals (—3, —2) were on the whole uncommon in the probands relatives.

GAMMAGLOBULIN IN RELATIVES KG

Earlier investigations of this pedigree had revealed unusually high gammaglobulin values in many members especially in siblings and in nephews and nieces of the proband (Leonhardt 1957 Larsson and Leonhardt 1959). Re-investigation of the siblings, nephews and nieces showed that these conditions had persisted (th other relatives were not included in this study) The distribution of the age-corrected gammaglobulin values among standardized intervals is shown in Table 42. Relatively more values in the first than in the second degree relatives were found to f ll within the + 2 and ≥ + 3 intervals; the difference was h wever not significant (χ = 0.10, DF = 3, P > 0 10). When all relatives of the proband KG were taken in one group the distribution of the gammaglobulin values for this group differed significantly from that of th controls A + B (χ^2 = 48.38, DF = 3, P < 0.001) There was, however also a difference between relatives of probands KG and the relatives of th other probands with definite SLE (= relatives A): the number of values falling within the + 2 and ≥ + 3 intervals in the former was higher than expected (χ = 18.02, DF = 3, P < 0 001). Th last comparison is n t quit valid, since the KG group" included both first and second degree relatives, relatives A consisting f only first degree relatives. Yet the u usually high frequency

Tabl 43. Distribution of the age-corrected alpha 2-globulin values in probands' relatives and in controls

Group	Se	Total tested	Standardized alpha-2-globulin intervals			
			0	+1	+2	≥+3
Relatives A	Males	115	59	43	8	5
	Females	110	52	41	10	7
	Total	225	111	84	18	12
	%		49.3	37.3	8.0	5 4
Relatives B	Males	111	47	45	14	5
	Females	106	52	38	10	6
	Total	217	99	83	24	11
	%		45.6	38.2	11 1	5.1
Controls A	Males	100	55	38	6	1
	Females	85	45	31	6	3
	Total	185	100	69	12	4
	%		54.0	37.3	6.5	2.2
Controls B	Males	80	34	35	6	5
	Females	59	30	23	3	3
	Total	139	64	58	9	8
	%		46.0	41.7	6.5	5.8

of gammaglobulin values belonging to the higher standardized intervals in pedigree KG gives reason to suspect special genetic or environmental factors.

ALPHA 2 GLOBULIN IN RELATIVES AND CONTROLS A+B

To ascertain whether the differences between probands' relatives and controls were true also for serum protein fractions containing only traces of immunoglobulins, the distribution of the alpha 2-globulin was studied. This also tended to vary with age and sex. The regression coefficient was calculated as 0 02 g per 100 ml per 10 year interval. The alpha 2 values were age-corrected and distributed among standardized intervals, as described above for gammaglobulin. The results are given in Table 43, and statistic analysis showed.

	χ^2	DF	P
Between relatives A and relatives B	1.45	3	>0.60
Between controls A and controls B	4 17	3	>0.20
Between relatives A and controls A + B	1.40	3	>0.70
Between relatives B and controls A + B	4.54	3	>0.20

Thus no significant differences were found between the various groups of respondents.

COMPARISON WITH PUBLISHED SERIES

Only a few authors ha e studied the quantitative variation of serum proteins in relatives of patients with SLE. Previous investigations by Leonhardt and by Larsson and Leonhardt have already been mentioned. Brunjes *et al* (1961) described

a family with 4 definite or suspected cases of SLE (Table 4) a few healthy family members had "hypergammaglobulinemia" as judged by the results of electrophoresis on paper Morteo *et al* (1961) found 8 (18 %) of 44 relatives (first *and* second degree) to have "hypergammaglobulinemia In the control material consisting of 38 healthy subjects and 8 with non-collagen diseases, 2 cases (4 %) of "hypergammaglobulinemia were f und. Of *asymptomatic* relatives (39 persons) only 4 (10 %) had hypergammaglobulinemia, a number not differing significantly from that found in the controls. In the evaluation, the results of both electrophoresis on paper and of the zinc turbidity test were used. Pollak *et al* 1960 who used the paper electrophoretic method, found no difference in the distribution of the gammaglobulin values in 50 relatives (first *and* second degree) of patients with SLE and 124 subjectively healthy controls. They did not mention any correction for age and sex. Holman and Deicher (1960), and Rodnan (1960) reported an increased frequency of hypergammaglobulinemia in SLE relatives, but the results were not given in detail. Finally Siegel *et al* (1961) using the paper electrophoretic method, found 16 cases of "hypergammaglobulinemia (defined according to the normal values of their laboratory) in 142 relatives of patients with SLE (11.3 %), while 142 age- and sex-matched relatives of patients with non-collagen diseases included 13 cases of "hypergammaglobulinemia" (9.2 %) thus no significant difference. When the gammaglobulin levels were judged according to a zinc turbidity method, however they found 27 cases of "hypergammaglobulinemia (19 %) in relatives of probands with SLE and 13 cases (9.2 %) in the controls, a significant difference ($\chi^2 = 4.92$, DF = 1 $P < 0.05$).

It deserves mentioning that hypergammaglobulinemia has been found in relatives of patients with acquired" agammaglobulinemia (Fudenberg *et al* 1962, Wolf *et al* 1963). Also monoclonal hypergammaglobulinemia has occasionally been encountered in such relatives (Lindholm 1959).

ANALYSIS OF RHEUMATOID FACTORS

METHOD

The tests for rheumatoid factors were performed in the Department of Clinical Bacteriology Malmö General Hospital.

In all 3 tests whole serum was used inactivated by immersion for 30 minutes in a 56° C water bath. Heterophile Absorbed Sheep Cell Test (SSC-test) was performed according to conventional techniques (Heller *et al* 1949 Winblad 1960). The FII Acryl Particle Fixation Test (FII AP-test) was performed as described by Winblad (1960). This test corresponds to the FII Latex Particle Test introduced by Singer and Plotz (1956) but the latex particles were substituted by polymetaacrylplast particles (Bofors) 0.8 μm in diameter The FII A Coated Tanned Sheep Cell Test (FIIA SC-test) was performed according to the principles given by Heller *et al* (1954) but, instead of native Cohn Fraction II, aggregated gammaglobulin was used (Epstein *et al* 1957) Aggregation was performed by heating the gammaglobulin solution at 63° C for 45 minutes (see Winblad 1965). In both FII AP and FIIA SC-tests, commercial FII (gammaglobulin KABI) was used.

The tests were done in the routine work of the laboratory and the *reproducibility* was controlled from day to day with positive and negative standard sera.

RHEUMATOID FACTORS IN PROBANDS

The results of the SSC- FII AP and FIIA SC-tests in probands are given in Appendix Tables II—IV A tendency to higher titres in probands B is apparent. In Tables 44—46 the results have been grouped under the headings of "negative "borderline and positive The titres corresponding to this classification are as follows:

Test	*negative*	*borderline*	*positive*
SSC	0	16—32	≥ 64
FII AP	0	10—40	≥ 80
FIIA SC	0	8—64	≥128

It follow from Tables 44—46 that probands A and probands B contain roughly the sam frequency of borderline results f th 3 tests fo RFs, but probands B contain many more positive results than probands A. Statistic analysis with adjustment for sex and age, gave the following results:

	χ^2	*DF*	*P*
Between probands A and probands B			
SSC	11.66	2	<0.01
FII AP	13.74	2	<0.01
FIIA SC	8.13	2	<0.05

It should be noted that the difference between the 2 groups of probands was least pronounced ($P < 0.05$) regarding the *FIIA-SC-test* which was else the most sensitive of the 3 tests for RFs employed.

The results of the tests for RFs in the probands were dependent on the clinical picture Probands with chronic polyarthritis,

Table 44. Distribution, among negative (—), borderline (±) and positive (+) results, of SSC-test in probands

Age (years)	Probands A				Probands B[1]			
	Total tested	SSC-test			Total tested	SSC-test		
		—	±	+		—	±	+
<45	18	10	5	3	13	4	1	8
≥45	19	12	4	3	22	8	4	10
Total	37	22	9	6	35	12	5	18
%		59.5	24.3	16.2		34.3	14.3	51.4

Males and females pooled

Table 45. Results of FII-AP-test in probands

Age (years)	Probands A				Probands B[1]			
	Total tested	FII-AP-test			Total tested	FII-AP-test		
		—	±	+		—	±	+
<45	18	12	5	1	13	4	2	7
≥45	19	13	2	4	22	6	4	12
Total	37	25		5	35	10	6	19
		67.6	18.9	13.5		28.6	1 .1	54.3

Males and females pooled

Table 46. Results of FIIA SC-test in probands

Age (years)	Probands A				Probands B[1]			
	Total tested	FIIA SC-test			Total tested	FIIA-SC-test		
		—	±	+		—	±	+
<45	18	8		3	13	4	3	6
≥45	19	9	6	4	22	4	6	12
Total	37	1	13		35	8	9	18
%		45.9	35.1	15.9		22.9	25.	51.4

Males and females pooled

Sjögren's syndrome and liver cirrhosis thus had much higher incidences of bor derline and positive results f all 3 tests than probands without such manifestations. Of 36 probands A — B *with* chronic polyarthritis *etc* 55.6 had positive SSC 61.2 % positive FII AP and 61.2 % positive FIIA SC-test. In the 36 probands A — B *without* such manifestations, the corresponding percentages were 11.1 5.5 and 11 1 % respectively. The differences found between th 2 groups of probands were evidently caused by the greater number of patients with chronic polyarthritis *etc* in probands B.

Borderline and positiv results of the

tests for RFs were much more common among probands than among controls, as is apparent from comparison of Tables 41—46 with Tables 47—49. Statistic analysis, with adjustment for age gave the following results (as the probands were nearly all females, only female controls have been implicited in the comparisons)

Between probands A and controls A + B	χ^2	DF	P
SSC	16.06	2	<0.001
FII-AP	29.49	2	<0.001
FIIA SC	30.33	2	<0.001
Between probands B and controls A + B			
SSC	61.35	2	<0.001
FII AP	67.28	2	<0.001
FIIA-SC	70.37	2	<0.001

RHEUMATOID FACTORS IN RELATIVES AND CONTROLS A+B

The distributions of the titres of the SSC-, FII AP and FIIA SC-tests in probands relatives and in controls are given in Appendix Tables II—IV. The highest titres were found in the groups of relatives, particularly in relatives B. In Tables 47—49 the results have been grouped under the headings of negative borderline and positive as above for the controls. Statistic analysis, with adjustment for sex and age gave as follows.

	χ^2	DP	P
Bet een relatives A and relatives B			
SSC	5.74	2	>0.05
FII AP	2.60	2	>0.20
FIIA-SC	0.51	2	>0.70
Between controls A and controls B			
SSC	5.54	2	>0.05
FII AP	3.61	2	>0.10
FIIA SC	2.50	2	>0.20
Between relati es A and controls A + B			
SSC	0.32	2	>0.80
FII AP	4.83	2	>0.05
FIIA SC	9.01	2	<0.05
Between relatives B and controls A + B			
SSC	12.52	2	<0.01
FII AP	4.87	2	0.05
FIIA SC	8.35	2	<0.05

From Tables 47—49 and from the above analysis the following conclusions can be drawn. Controls B contained numerically more borderline and positive results of the 3 tests for RFs than controls A, in no instance however were the differences statistically significant. Relatives B had somewhat higher percentages of borderline and positive results of SSC-test than relatives A, but the difference between the groups of relatives was not significant. As for th other tests for RFs, no systematic differences were discernible. There was an obvious difference between relatives A and controls only regarding the FIIA SC-test, for which relatives A showed a higher incidence of borderline values. On comparison between relatives B and controls, on the other hand a systematic tendency to higher incidences of borderline as well as of positive results of all 3 tests for RFs was evident.

As a general remark it should be pointed out that borderline and positive results of the tests for RFs were more common in the higher age classes of relatives as well as of controls (Tables 47—49). But there were no consistent sex differences. This is in agreement with common experience (see e g Ball and Lawrence 1961)

RHEUMATOID FACTORS AND SUBJECTIVE STATE OF HEALTH

Twelve relatives A and 8 relatives B had gross collagen or autoimmune diseases known to be associated with positive tests for RFs (Table 27 page 67). Two controls had chronic polyarthritis (Table 28, page 68). Exclusion of these cases will decrease the incidences of borderline and positive results of tests for RFs in relatives (A as

Table 47. Results of SSC-test in probands' relatives and in controls

Sex	Age (years)	Relatives A: Total tested	SSC test —	SSC test ±	SSC test +	Relatives B: Total tested	SSC test —	SSC test ±	SSC test +	Controls A: Total tested	SSC-test —	SSC-test ±	SSC-test +	Controls B: Total tested	SSC-test —	SSC-test ±	SSC-test +
Males	< 15	62	60	0	3	56	44	10	2	47	43	2	2	36	20	7	0
	≥ 15	53	46	1	3	55	42	11	2	51	46	3	2	44	36	6	2
	Total	115	96	13	6	111	86	21	4	98	89	5	4	80	65	13	2
	%		83.5	11.3	5.2		77.5	18.9	3.6		90.8	5.1	4.1		81.3	10.3	2.5
Females	< 45	57	50	7	0	59	40	14	5	55	50	5	0	23	20	1	1
	≥ 45	53	45	6	2	46	35	7	1	30	26	2	2	37	29	7	1
	Total	110	95	13	2	105	75	21	9	85	76	7	2	60	49	8	2
	%		86.4	11.8	1.8		71.4	20.0	8.6		89.4	8.2	2.4		83.1	13.0	3.1

Table 48. Results of FII AI test in probands relatives and in control

Sex	Age (years)	Relatives A: Total tested	FII AP test —	FII AP test ±	FII AP test +	Relatives B: Total tested	FII AP test —	FII AP test ±	FII AP test +	Controls A: Total tested	FII AP test —	FII AP test ±	FII AP test +	Controls B: Total tested	FII AP test —	FII AP test ±	FII AP test +
Males	< 15	62	60	2	0	56	53	2	1	49	46	3	0	36	36	0	0
	≥ 15	53	51	2	0	55	50	3	2	51	50	0	1	44	40	2	2
	Total	115	111	4	0	111	103	5	3	100	96	3	1	80	76	2	2
	%		96.5	3.5	0.0		92.8	4.5	2.7		96.0	3.0	1.0		95.0	2.5	2.5
Females	< 45	57	54	2	1	60	53	2	5	55	55	0	0	22	20	2	0
	≥ 45	53	44	7	2	46	40	2	1	30	30	0	0	37	31	0	3
	Total	110	98	9	3	106	93	4	9	85	85	0	0	59	51	2	3
	%		89.1	8.2	2.7		87.7	3.8	8.5		100.0	0.0	0.0		91.5	3.4	5.1

Table 51 Results of tests for RFs in relatives KG

Group[1]	Total tested	SSC-test			FTI AP-test			FIIA SC-test		
		−	±	+	−	±	+	−	±	+
Brothers	3	3	0	0	3	0	0	2	1	0
Sisters	8	4	3	1	5	1	2	3	2	3
Nephews	19	19	0	0	19	0	0	16	3	0
Nieces	19	16	3	0	17	1	1	13	5	1
Total	49	42	6	1	44	2	3	34	11	4
%		65.7	12.3	2.0	89.8	4.1	6.1	69.4	22.4	8.2

[1] All ages

tions by Leonhardt and by Larsson and Leonhardt have already been referred to (page 22). Holman and Deicher (1960) as well as Rodnan *et al* (1960) observed RFs in SLE relatives; no details were however reported. Morteo *et al* (1961) found positive reactions for RFs in 16 (36.4 %) of 44 SLE relatives. Fourteen were asymptomatic, while 2 had "classic rheumatoid arthritis". RFs were measured "by the latex fixation test, the capillary latex test, and by the sheep cell agglutination and inhibition tests of Ziff carried out with englobulins prepared from serum. Only 4 (8.7 %) of 46 controls had positive tests for RFs. In the series of Siegel *et al* (1961), 12 (8.5 %) of 142 relatives and 9 (6.3 %) of 142 controls were found to have positive "latex fixation test" thus no significant difference. According to their preliminary communication Ansell and Lawrence (1963) found no increased frequency of RFs in SLE relatives.

As far as the SLE-like syndromes are concerned, detailed investigations have as yet been made only in *rheumatoid arthritis*. An increased incidence of RFs in relatives of patients with RA has been reported by Lawrence and Ball (1958), Ziff *et al* (1958), Bremner *et al* (1959) and De Blécourt *et al* (1962). The increased incidence of RFs seems, however, to be limited to the relatives of sero-positive probands, the incidence in relatives of sero-negative probands not being higher than that in controls (Lawrence and Ball 1958).

A tendency to higher incidence of RFs in relatives of patients with *Sjögren's syndrome* was preliminary reported by Burch *et al* (1963).

CHAPTER 9

ANALYSIS OF ANTINUCLEAR FACTORS

METHOD

Antihuman gammaglobulin serum was produced and labelled with fluorescein isothiocyanate by the author using the facilities of the Institution for Clinical Bacteriology Malmö General Hospital. The preparations for demonstration of antinuclear factors were made in the author's laboratory in the Royal Dental School of Malmö by a specially trained technician. The specimens were examined for nuclear fluorescence personally by the author

For the demonstration of ANFs, a modification of Coons indirect fluorescent antibody method was used (Coons *et al* 1941), described by Friou *et al* (1957) Holborow *et al* (1957) and Holman and Kunkel (1957). Rabbits were immunized with commercial human gammaglobulin (Cohn s fraction II, KABI). The rabbit serum globulin fraction obtained by precipitation with ammonium sulphate was conjugated with fluorescein isothiocyanat (Behringwerke) in the ratio f 0 05 mg/1 mg protein. The conjugate was dialysed against phosphate buffer and then absorbed with dry Sephadex G 25 medium (Pharmacia) as described by Bien (1962). Th resulting conjugate was dil ted 1:32. In this dilution the con jugate still gav strong nuclear fluorescence with a control SLE serum and the amo nt of unspecific fluorescence was unimportant.

Human leucocytes were used as a substrate for binding ANFs. Blood films were prepared from one 0 Rh-positive donor throughout the in estigation. They were treated with the sera *to be tested* and then with the fluorescent conjugate in the way described by Hijmans *et al* (1962)

Fluorescent anti-human-globulin of on and the same batch was used throughout this investigation. Immunoelectrophoretic analysis (performed at the Laboratory for Clinical Chemistry Malmö General Hospital) showed that this conjugate contained antibodies against 7S as well as 19S globulins.

The preparations were examined in a Zeiss microscope adapted for fluorescence. An Osram HBO 200 mercury lamp was used as a source of light. Dark field illumination was obtained through a Zeiss Ultracondensor As primary filters use was made of Schott UG 5 and BG 12, as a secondary filter GG 9

The degree of nuclear fluorescence in positive preparations was graded from + to ++++ In ++++ preparations, the nuclei showed strong fluorescence at the place of the nuclear membrane. In +++ and ++ preparations, the nuclei showed a homogenous fluorescence. In + preparations, the fluorescence was weak and the nuclei were usually swollen, often extruding beyond the cell boundaries. Preparations with fluorescence material out side the cell membrane only but so ar ranged as t suggest nuclear origin were designated ± (doubtfully positive). All ther preparations were considered negative (—). This scale of positivity was repeatedly reproduced by dilution of sera with positive LE-cell phenomenon.

The reproducibility f the method was

Table 54 Results of fluorescence test for ANFs in relatives KG

Group[1]	Total tested	Test for ANF		
		—	±	+
Brothers	3	3	0	0
Sisters	8	6	2	0
Nephews	19	19	0	0
Nieces	19	19	0	0
Total	49	47	2	0
%		95 9	4 1	0 0

All ages

Thus the distribution of the results of test for ANFs in relatives A as well as in relatives B differed significantly from that in the controls. Relatives A showed a higher percentage of *positive* results than relatives B the difference was not statistically significant but might nevertheless reflect the above mentioned tendency to higher degree of positivity in probands A than in probands B

It is evident from Table 53 that the incidence of positive and doubtfully positive results of test for ANFs in the male relatives was lower throughout than in the female relatives. But there was no difference with age

ANTINUCLEAR FACTORS AND SUBJECTIVE STATE OF HEALTH

Exclusion of the 12 relatives A and 8 relatives B with gross collagen or autoimmune diseases (Table 27) and of the 2 controls with chronic polyarthritis (Table 28) did not affect the results described above.

ANTINUCLEAR FACTORS IN RELATIVES KG

The results of the fluorescence test for ANFs in relatives of proband KG are given in Table 54 There was a remarkable lack of positive results in the relatives. Only 2 relatives, both sisters of the proband had doubtfully positive results. Thus the distribution differed not only from that of the relatives A and relatives B but also from that of the controls In all these groups the frequency of borderline and positive results was higher than in relatives KG

Special mention should be made of the fact that ANFs could *not* be demonstrated in the following cases, all in sisters of the proband KG (see Figure 11) the 60-year-old sister (II.3 in Figure 11) who had had transient CDLE of the face the 44 year-old sister (II 11) who had suspected SLE, the 41 year-old sister (II 12) who had had widespread CDLE, and the 39-year-old sister (II 13) with essential" hypergammaglobulinemia (see also Chapter 13)

COMPARISON WITH PUBLISHED SERIES

The incidence of positive fluorescence tests for ANFs in *SLE patients* approaches 100 in the various series on record (see Hijmans 1963). When titrations of the ANFs were performed, patients with SLE were found to have higher titres

than othe categories of patients (see *e g* Baugh *et al* 1960) The incidence f positive tests for ANFs in *patients with RA* is considered to be rather high altho gh the percentages vary (from 10 to 65 %, see Hijmans 1963) with the sensitivity of the method used and probably also with the composition of th patient material. In healthy controls 0—4 % positive results and in diseased controls 1—15 % posi tive results have been reported. The results obtained by the present writer with sera from patients with SLE, and from controls agree in principal traits with those reported in the literature.

The incidence of ANFs in *SLE relatives* varies considerably in the few series on record. Th highest incidence, 48.0 % (of 50 relatives), was reported by Pollak *et al* (1961). In a larger series (Pollak 1963) they found positive tests in 47 (33.1 %) of the 142 first degree and in 10 (21.3 %) of the 47 second degree relatives studied. Fennell *et al* (1962) stated that 38 (42.2 %) of 90 SLE relatives had ANFs. Morteo *et al* (1961) reported 6 (14 %) of 44 Siegel *et al* (1961) 7 (4 9 %) of 142 and Ansell *et al* (1963) 4 (4 9 %) of 81 SLE relatives to have positive fluorescence tests for ANFs. Most of these reports were preliminary communications. The wide variations in the incidences of positive tests might be dependent partly on differences in selec tion of probands and relatives and partly on varying techniques for the demonstration of ANFs, including different properties of the various fluorescent conjugates used (Hijmans *et al* 1963). Although the incidences of positive tests vary from author to au thor all agree that positive tests are more common among SLE relatives than among controls. In the present material the dif ferences between relatives of patients with SLE, definite or suspected and controls was very clear

ANALYSIS OF SEROLOGIC REACTIONS FOR SYPHILIS

METHOD

The serologic tests for syphilis were performed in the Department of Clinical Bacteriology Malmö General Hospital. The tests were done according to the usual methods, including complement fixation with cardiolipin (Wassermann s reaction) and Meinicke s and Kline s cardiolipin reactions.

In those sera reacting positively in any of the 3 tests, the TPI test was performed at *Statens Bakteriologiska Laboratorium* Stockholm.

SEROLOGIC REACTIONS FOR SYPHILIS IN PROBANDS

Four (10.8 %) of the 37 probands A tested had positive STS. Two of them had a positive reaction in all 3 tests, though in a relatively low titer two had only positive Meinicke-reaction. All had had positive reactions for syphilis during previous spells in hospital and check examination with TPI had proved negative The reaction had remained positive for more than 6 months, and these cases may thus be regarded as "chronic false-positive reactors" (Shulman 1963). The remaining 33 probands of group A tested in the present study reacted negatively in all 3 tests; two of these had previously shown positive reactions.

Only 1 (2.9 %) of the 35 probands B tested for the present investigation gave a positive reaction and then in all 3 tests. This proband had also previously shown repeatedly positive reactions despite a negative TPI test and may be regarded as a chronic false-positive reactor Another 4 group B probands still living at the time of study had previously showed positive STS but when tested for the present investigation they were negative.

An *anticomplementary phenomenon* was found in 2 group B but in no group A probands when their sera were examined with Wassermann s reaction. One of the 2 probands B with anticomplementary activity had during previous spells in hospital been reported to have clearly positive STS and a negative TPI

Analysis of the hospital records of the probands (see Chapter 3) had revealed an incidence of 15 % positive STS in group A probands as well as in group B probands. At the present after-examination the percentage was lower 11 % for group A and 3 % for group B probands. This decrease was, of course due to a larger number of investigations on different occasions being included in the former calculations; the latter percentages were calculated from one examination only The activity of the SLE may possibly also play a role (Zellman 1952)—samples for the present investigation were usually taken during periods of remission.

SEROLOGIC REACTIONS FOR SYPHILIS IN RELATIVES AND CONTROLS A+B

Of the 324 *controls* tested, all showed negative STS.

Of the 225 *relatives A* only 2 reacted positively One was the 43-year-old brother of proband 11 He proved strongly positive in all 3 tests, check-examination with TPI showed that it was a question of true positive reactions for syphilis, and the respondent admitted the possibility of previous infection. The other case was the 63-year-old mother of proband 21. A weakly positive Meinicke reaction was found, the other tests for syphilis were negative, as was the TPI. The patient had chronic deforming polyarthritis and showed at the interview suspected CDLE of the face.

Five of the *relatives B* tested (2.3 %) had one or more positive STS. In all these cases the TPI-reaction was negative, so that the positive reactions for syphilis may be regarded as non-specific. Two of the false-positive reactors, a 63-year-old female with suspected SLE and a 65-year old subjectively healthy male were both siblings of proband 95 who had previously been known as a chronic false-positive STS reactor This pedigree will be described in Chapter 13 (see also Figure 8). Another female relative of group B with false-positive STS, the 57 year-old sister of proband 106—previously repeatedly shown to have had false-positive STS—will also be described in Chapter 13, as a case of "essential" hypergammaglobulinemia (see also Figure 10). The 4th of the false-positive reactors, the 45-year-old mother of proband 103, had epileptiform attacks (see also Chapter 13). The last member of relatives B with false-positive STS, the 30-year-old son of proband 107 was subjectively healthy

SEROLOGIC REACTIONS FOR SYPHILIS IN RELATIVES KG

All 11 first degree and 38 second degree relatives of proband KG examined with STS reacted negatively

COMPARISON WITH PUBLISHED SERIES

The incidences of false-positive STS in *SLE patients* according to the literature were given in Chapter 1 (Table 2).

Positive STS in *SLE relatives* have been reported by Rodnan *et al* (1960), who, however did not give any detailed data. Morteo *et al* (1961) reported 5 (11 %) cases of false-positive STS among 44 SLE relatives. All 5 were subjectively healthy but 3 also had hypergammaglobulinemia and a positive test for RFs. Morteo *et al* used a battery of 5 different tests for syphilis, and by the TPI-test they excluded a specific cause in the positive cases. In their preliminary study Siegel *et al* (1961) reported that 5 (3.5 %) of 142 relatives of SLE patients gave false-positive reactions for syphilis, none of the corresponding number of controls reacted positively They employed a macroflocculation test for syphilis, using cardiolipin-lecithin antigen.

In the present material the number of false-positive STS in relatives of probands was small. In only 1 of group A and 5 of group B relatives were false-positive reactions noted. Two of the false-positive reactors were siblings, and the corresponding proband also had a false-positive STS. Another female was the sister of a proband who had earlier been a chronic false-positive reactor In association with these examples of familial occurrence of false-positive STS it should be mentioned that this phenomenon has been observed in a family without evidence of collagen diseases (Cannon 1958).

ANALYSIS OF ANTISTREPTOLYSIN

METHOD

Antistreptolysin O was determined as described by Ingestad and Winblad (1963). All sera were tested in the routine of the Department of Clinical Bacteriology Malmö General Hospital.

Among other sera, blood donor sera had been studied in the Department of Bacteriology continually over a period of 5 years, the results were reported by Ingestad and Winblad (1963). Of 2040 serum specimens, 81.3 % showed ASL titres below 300 units, ASL values of 300 units or more were therefore regarded as elevated. There was a clear seasonal variation, the positive titres being most prevalent during February to July

ANTISTREPTOLYSIN IN PROBANDS

The distribution of results of test for ASL is given in Table 55 They have been grouped under the headings of "negative" (titres 0—125 U) borderline" (150—250 U) and "positive" ($\geq$ 300 U) The ASL titres of the probands were apparently little influenced by the season when the blood specimens had been drawn which was therefore ignored. It follows from Table 55 that probands A had a higher incidence of positive ASL titres than probands B. The distributions among results of test for ASL in the 2 groups of probands differed significantly ($\chi = 6.60$ DF $= 2$, $P < 0.05$).

Probands A had a higher incidence of positive results of ASL test than the controls (since nearly all probands were females, the comparisons should be made with the *female* controls). The results of ASL testing in probands B on the other hand, were largely the same as in the female controls (Table 56) Statistic analysis, with adjustment for age gave as follows.

	χ^2	DF	P
Between probands A and female controls A + B	7.93	2	< 0.05
Between probands B and female controls A + B	0.43	2	> 0.80

Table 55 Distribution, among negative (—), borderline ($\pm$) and positive (+) results, f test for ASL in probands

Age (years)	Probands A[1]				Probands B			
	Total tested	Test for ASL			Total tested	Test for ASL		
		—	$\pm$	+		—	$\pm$	+
<45	18	6	8	4	13	8	3	2
≥45	19	3	4	12	22	9	7	6
Total	37	9	12	16	35	17	10	8
		24.3	32.4	43.3		48.6	28.6	22.8

[1] Males d f males pooled

Table 56. Results of test for ASL in probands' relatives and in controls

Sex	Age (years)	Season	Relatives A				Relatives B				Controls A				Controls B			
			Total tested	Test for ASL			Total tested	Test for ASL			Total tested	Test for ASL			Total tested	Test for ASL		
				—	±	+		—	±	+		—	±	+		—	±	+
Males	< 15	Febr—July	29	7	9	13	9	2	3	4	23	7	9	7	12	3	1	8
		Aug—Jan	33	14	6	13	47	27	16	14	26	5	9	12	24	3	9	12
	≥ 15	Febr—July	28	15	7	6	6	3	1	2	21	8	5	8	8	2	3	3
		Aug—Jan	25	10	10	5	49	17	21	11	30	9	7	14	36	16	6	14
	Total		115	46	32	37	111	39	41	31	100	29	30	41	80	24	19	37
				40.0	27.8	32.2		35.1	37.0	27.9		29.0	30.0	41.0		30.0	23.8	46.2
Females	< 15	Febr—July	36	7	14	15	15	4	5	6	19	10	4	5	2	0	2	0
		Aug—Jan	21	7	6	8	45	11	18	15	38	12	18	8	20	11	2	7
	≥ 15	Febr—July	26	6	8	12	4	3	1	0	14	9	4	1	1	0	1	0
		Aug—Jan	27	6	12	9	42	17	11	14	16	7	4	5	36	14	15	7
	Total		110	26	40	44	106	35	35	36	85	38	28	19	59	25	20	14
	%			23.6	36.4	40.0		33.0	33.0	34.0		44.7	32.9	22.4		42.4	33.9	23.7

When blood samples had been draw

ANTISTREPTOLYSIN IN RELATIVES AND CONTROLS A+B

The results of test for ASL in probands relatives and in controls are given in Table 56. It is seen that of 321 controls tested (males + females) 111 or 34.3 % had positive titres (300 U or more). This incidence is considerably higher than that found by Ingestad and Winblad in 2016 blood donor's sera only 18.7 % of these had positive titres. The present authors control material included, however subjectively healthy as well as subjectively sick individuals, and the age and sex distributions were quite different as the majority of blood donors were men between 20 and 40 years of age.

From Table 56 it would appear that the incidence of positive titres is higher in male controls than in female controls, the percentage in the combined control material (controls A + controls B) being 43.3 % for men and 22.9 % for women. In Table 56 the results were classified according to sex, age and season when the blood specimens had been drawn. As the seasonal variation seemed to be small, this variation was ignored in the statistic evaluations which follow. It is remarkable that in the *relatives* the sex dependence seemed to differ from that in the controls, the highest percentages of positive results were found in the *female* relatives. On comparison of the groups of relatives with each other and with the controls, the following statistic results (after adjustment for age and sex) were obtained.

	χ^2	DF	P
Between relatives A and relatives B	0.79	2	>0.50
Between controls A and controls B	0.57	2	>0.70
Between relatives A and controls A + B	0.74	2	>0.60
Between relatives B and controls A + B	1.21	2	>0.50

Thus, no significant differences between the various groups of respondents could be demonstrated. However when each sex was treated separately some trends of interest appeared.

	χ^2	DF	P
Between male relatives A and male controls A + B	5.44	2	>0.05
Between female relatives A and female controls A + B	13.81	2	<0.001
Between male relatives B and male controls A + B	8.35	2	<0.05
Between female relatives B and female controls A + B	3.68	2	>0.10

The most remarkable result of treating each sex separately was the demonstration of a significant difference between female relatives A and female controls, the former having a higher frequency of positive (and borderline) values.

ANTISTREPTOLYSIN IN RELATIVES KG

The distribution of the results of ASL testing in the relatives of probands KG is shown in Table 57. It is seen that these relatives had a very high incidence of positive results—of 49 relatives, 30 or 61.2 % were ASL-positive. This incidence appears higher than that in relatives A or in the controls. When men were compared with men and women with women, the following results were obtained (no regard has been taken to age groups, judging from the literature the ASL titres tend to decrease with age and the greater proportion of respondents 45 years of age or more among relatives A than among controls would tend to decrease the differences in comparisons with relatives KG):

	χ^2	DF	P
Between relatives KG and relatives A	13.73	2	<0.01
Between relatives KG and controls A + B	19.62	2	<0.001

Table 57 Results of test for ASL in relatives KG

Group[1]	Total tested	Test for ASL		
		−	±	+
Brothers	3	0	2	1
Sisters	8	0	2	6
Nephews	19	2	3	14
Nieces	19	3	7	9
Total	49	5	14	30
%		10.2	28.6	61.2

[1] All ages

Thus there was a significant difference between relatives of proband KG and relatives A, the difference between relatives KG and controls was significant

COMPARISON WITH PUBLISHED SERIES

Reports of ASL in *patients with SLE* have been mentioned in Chapter 1 (page 13) In the present series, probands A appeared to have a particularly high incidence of positive titres.

ASL was investigated previously in pedigree KG (Leonhardt 1959) the titres were then apparently high, as they still were in the present study Other reports of ASL in *SLE relatives* have not been published.

SUMMATION OF PHYSICAL AND LABORATORY ABNORMALITIES IN RELATIVES AND CONTROLS

RELATIONSHIPS BETWEEN LABORATORY VARIABLES

In screening the relationships between the serologic variables, classification of the respondents according to age and sex was not considered necessary Moreover in the following tables (Tables 58—61) probands' relatives and controls were pooled. Analysis of each group separately did not suggest that relatives and controls differed in any way regarding the relationships studied.

BLOOD GROUPS—GAMMAGLOBULIN AND SEROLOGIC FACTORS

In Table 58, the results of gammaglobulin determination and of FIIA SC- ANFs- and ASL tests were subdivided according to blood groups. An association between blood groups and the other variables was found only for FIIA SC-test ($P < 0.05$). In this test there was among other things a decreasing frequency of blood group A with increasing positivity of the results. The finding was, however believed to be irrelevant.

GAMMAGLOBULIN—SEROLOGIC FACTORS

In Table 59 the results of FIIA SC-test and test for ANFs were grouped according to distribution among standardized gammaglobulin intervals. A tendency to higher gammaglobulin levels with increasing positivity of the results was seen throughout The differences were statistically significant.

Gammaglobulin in relation to	χ^2	*DF*	*P*
FIIA SC	33.39	6	<0.001
ANFs	22.18	6	<0.01
ASL	14.40	6	<0.05

RHEUMATOID FACTORS—ANTINUCLEAR FACTORS AND ANTISTREPTOLYSIN

The results of tests for ANFs and ASL were grouped according to the degree of positivity in the FIIA SC-test (Table 60) This test for RFs was chosen as the most sensitive one.

More borderline and positive results of FIIA SC-test were found in the ANFs ± and ANFs + than in the ANFs — respondents. No association was found between ASL and RFs.

FIIA SC-test in relation to	χ^2	*DF*	*P*
ANF	32.48	4	<0.001
ASL	0.09	4	>0.95

ANTINUCLEAR FACTORS—ANTISTREPTOLYSIN

In Table 61 the results of ASL testing were grouped according to the degree of positivity in the test for ANFs. No significant association was found

ANF in relation to	χ^2	*DF*	*P*
ASL	6.46	4	>0.10

Table 58. Relationship between ABO blood groups and gammaglobulin levels, results of FIIA SC test, of test for ANF and for ASL in respondents. In this and in the following tables (Tables 59—61), the probands' relatives and controls have been pooled, irrespective of main group (A or B), sex and age. The gammaglobulin levels have been age-corrected and distributed among standardized intervals, and the results of the serologic tests have been expressed as negative (—), borderline or doubtfully positive (±) and positive (+)

Test	Class of result	Number in class	Distribution among blood groups							
			A		B		O		AB	
			No.	%	No.		No.	%	No.	%
Gammaglobulin	0	334	162	48.5	29	8.7	133	39.8	10	3.0
	+1	266	128	48.1	26	9.8	94	35.3	18	6.8
	+2	105	53	50.4	13	12.4	34	32.4	5	4.8
	≥+3	48	17	35.4	5	10.4	23	47.9	3	6.3
	All classes	733	360	47.8	73	9.7	284	37.7	36	4.8
FIIA SC	—	597	294	49.2	52	8.	222	37.2	29	4.9
	±	113	49	43.3	14	12.4	48	48.5	2	1.8
	+	37	12	32.4	7	18.9	13	35.2	5	13.5
	All classes	747	355	47.5	73	9.8	283	37.9	36	4.8
ANF	—	627	297	47.4	58	9.2	240	38.3	32	5.1
	±	57	27	47.4	8	14.0	19	33.3	3	5.3
	+	64	31	48.4	7	10.9	25	39.1	1	1.6
	All classes	748	355	47.4	73	9.8	284	38.0	36	4.8
ASL	—	267	134	50.2	28	10.5	93	34.8	12	4.5
	±	235	111	47.2	19	8.1	91	38.7	14	6.0
	+	251	114	45.4	27	10.8	100	39.8	10	4.0
	All classes	753	359	47.7	74	9.8	284	37.7	36	4.8

SEROLOGIC REACTIONS FOR SYPHILIS—OTHER SERUM PROTEIN FACTORS

Owing to the smallness of the number of positive results of STS, these were not studied statistically for any association with other laboratory variables. The clinical state of the false-positive STS-reactors has been described in chapter 10 (page 101).

SUMMARY OF RELATIONSHIPS BETWEEN LABORATORY VARIABLES

The relationships between the laboratory variables studied have been summarized in Table 62. It is apparent that elevated gammaglobulin levels and positive tests for RF and ANFs were often combined. Thus often more than one of the serologic abnormalities of "autoimmune" type (hypergammaglobulinemia, RFs, ANF) were present in the same individual, who might have a constitutional "weakness" of the immunologic system (see Chapter 14 General Discussion).

No association was found between ASL and the other serum protein variables except gammaglobulin ($P < 0.05$). Thus the production of ASL—representing antibodies to exogenous antigens—might be dependent on factors other than those producing RF and ANFs. The ASL titres

CDLE and eruptions caused by light sensitivity may show external similarities. The cutaneous lesions in this particular case had however been considered as typical of light sensitivity by the dermatologists. The patient reacted also negatively in the fluorescence test for ANFs. According to Peterson and Fusaro (1963), this test aids in the differential diagnosis between CDLE and light sensitivity being negative in the latter condition.

SLE LIKE SYNDROMES IN RELATIVES A

PEDIGREE OF PROBAND 1

The proband's history was described in Chapter 3 (page 58). Among the protean manifestations of this woman, who died at the age of 38 years, were Coombs' positive anemia and thrombocytopenic purpura.

The *mother* of the proband, born 1890, developed in *1950* anemia (Hb 10 g per 100 ml), leucopenia (WBC 2600—4500) and a palpable spleen. The thrombocyte count was 160,000 and the ESR 40 mm/1 hour. In *1951* severe splenomegaly was observed. The reticulocytes were 2 % of the RBC and bilirubin in serum 2.2 mg per 100 ml. Splenectomy was performed, and at operation liver biopsy was also done. Microscopic examination of the spleen showed unspecific changes and the liver showed signs of mild hepatitis. The Hb increased to 13 g per 100 ml after operation, and the WBC was normal, but the ESR was still increased (60 mm/1 hour).

She was then in a good general condition until *1952*, when she had fever and was jaundiced. Thrombosis of the left leg supervened. An acute hemolytic crisis was diagnosed: Hb 6.1 g per 100 ml, RBC 1.7, serum bilirubin 8.8 mg per 100 ml, reticulocytes 60 % of the RBC. She now had leucocytosis, WBC 40,000. Coombs' direct test was negative on 2 occasions but positive on 2 others. Electrophoresis showed hypergammaglobulinemia (no figures). She was treated with cortisone and the hematologic picture improved. Half a year later she had another severe hemolytic crisis with RBC sinking to nearly 1 million per cmm. She had a leukemoid reaction with WBC, maximum 142,000. The initial thrombocyte counts were normal (359 000—192,000), but during treatment for pleuropneumonia with sulfonamides the values temporarily decreased to 52,000. Coombs' test was again positive. In the urinary sediment numerous granulated casts were repeatedly demonstrated. The patient was treated with cortisone and with blood transfusions. One transfusion in January *1953* was followed by gross hemoglobinuria. The cardiac state of the patient, who also had arterial hypertension, deteriorated and she had attacks of atrial fibrillation. NPN rose to 73 mg per 100 ml. In March 1953 the patient died.

Autopsy showed signs of arterial hypertension, small adhesions in both pleurae, hyperplastic bone-marrow and excessive hemosiderosis of the inner organs. No signs of SLE were recorded. Also on re-examination of the biopsy and necropsy specimens in 1963 (Prof C-G Ahlström), no signs of SLE were found. Moderate focal hepatitis was noted.

The proband's mother thus had *"idiopathic" hemolytic anemia*, leucopenia and splenomegaly; thrombocytopenia, drug-induced? pleuropneumonia, nephritis and focal hepatitis.—LE-cell tests had never been performed on this patient, who might well have had *SLE*.

Of the proband's 2 *daughters* 1 had a dubiously positive test for ANFs at the author's examination. This 18-year-old girl had also a borderline SSC-test (16). The 38-year-old *brother* of the proband also had a borderline SSC-test (16).

PEDIGREE OF PROBAND 5

This pedigree had been described previously by Larsson and Leonhardt (19 9). The 31 year-old *proband* died in a nephrotic syndrome with uremia and *post mortem* showed *inter alia* wire-loop lesions of the kidneys. She had had arthralgia and slight arthritis.

The *mother* died 63 years old from a severe illness with hypertension, cardiac and renal insufficiency, hemoptysis, roentgenologic lung densities, and increased ESR (70—29 mm/1 hour). Her history was reminiscent of SLE but the diagnosis was not verified.

The 39-year-old *sister* of the proband has chronic polyarthritis, and at the age of 31 years she had acute *SLE* with in

volvement of the heart, lungs, kidneys and blood cells, and positive LE-cell phe nomenon. She has since suffered only from her joint affection and at several examinations she has always shown hypergammaglobulinemia.—At the time of th present study in 1961 her general condition was good. She still had hypergamma globulinemia (1.5 g per 100 ml), the SSC test was borderline (32) but the FII AP and FIIA SC-tests positive (80 and 1024 respectively), and the test f ANF was ++ positive.—The *monozygotic twin* of the last patient had on the whole been healthy and in 1961 she was still in good health. Her previously demonstrated hypergammaglobulinemia persisted, however (1 7 g per 100 ml), and she had a + positive test for ANFa. The tests for RFs were uniformly negative (in 1959 the patient had had a borderline value of the SSC-test, 32)

The eldest (43-year-old) sister in this sibship has *deforming rheumat id arthritis*. Her condition had remained unchanged until 1961 wh n re-examined for the present study The gammaglobulin value was 1.2 g per 100 ml. She had a positive FIIA SC-test (128) and the fluorescence test for ANFs was ++ positive.

A distant *female relative* of proband 5 (see Larsson and Leonhardt 19.9) was included in the present material as having *suspected SLE* (proband 93).

PEDIGREE OF PROBAND 9

The case history of th *proband* was given in Chapte 3 (page 55). The course was protracted with several classic manifestations f SLE. She also had an attack of Ileitis and developed severe stricture f th esophagus reminiscent f systemic sclerosis.

The 4 year-old *mother* of the proband (Figure 5, I.2) underwent clinical examination t the Department of Internal Diseases in Malmö, electrophoresis in connection with the present investigation having shown pronounced hyper gammaglobulinemia (2 0 g per 100 ml). She had had arthralgia for t least 10 years and for many years an increasing feeling of stiffness of the

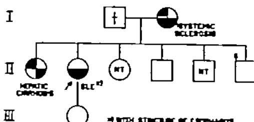


Figure 5. Pedigree of proband 9

fingers and ttacks of acrocyanosis in the cold. On examination she had slight dyspnea and slight pretibial edema. The skin of the limbs was tight and glossy most pronounced peripherally There was spindle-shaped swelling of the proximal interphalangeal joints bilaterally and capsular swelling of the radiocarpal joints. The thyroid was enlarged and adenomatous. Physical examination of the heart revealed signs of mitral stenosis and insufficiency X-ray showed generalized enlargement (520 crn³/m³ body surface) of the heart. Basal changes were found bilaterally in the lung fields and were ascribed to prev ious pleurisy Roentgen examination of the hands showed moderate periarticular rarefaction and erosion of the cartilage and bone of the small joints. Routine blood and urine examinations revealed nothing abnormal, but the ESR was 23 mm/1 hour and electrophoresis still showed hypergammaglobulinemia (2.1 g per 100 ml). A LE-cell test showed some free bodies but no LE-cells. Bromsulphalein test gave 10 % retention after 30 minutes. The diagnosis of scleroderma was confirmed by dermatologist, but biopsy of the skin showed only slight atrophy (the piece of skin was, however removed from proximal part of one leg).—ANF were sought for the present study and the result was +++ positive The SSC- and FIIA SC- tests were borderline (16 and 64 respectively).

Descriptive diagnosis of proband's mother Chronic polyarthritis. Scleroderma with Raynaud s phenomenon. Rheumatic heart disease. *Systemic sclerosis?*

The older sister of the proband (II.1 in Figure 5), 55-year-old woman, had periodically had arthralgia since the age of 37 years, and in *1938* she was then 45, she had nodose erythema of the lower legs together with bout of polyarthritis. In *1951* she was found to have thyroid insufficiency and was placed on substitution therapy In the same year she was operated upon because of myomata of the uterus, and in 1952 the left eye was extirpated because of neurinoma. *1957* the patient noticed yellow discolora-

tion f the skin and she began t be troubled by itching of the skin. In *1959* the liver was enlarged on palpation, and the spleen was roentgenologically slightly enlarged. She had slight icterus and some spiders were noted. Liver biopsy verified th diagnosis of liver cirrhosis (portal type). Incid ntally left-sided hypernephroma was discovered and removed t operation the sam year During hospitalization, the patient had spell of drug exanthema. Repeated electrophoretic examination of the serum proteins in 1959 bowed gammaglobulin alues consistently above 2.0 g per 100 ml, nd the albumin fraction was lowered. *No search was made for LE-cells.* During the f llowing 2 years the patient was severely troubled by f tigu itching of the skin and pronounced anemia necessitating repeated blood transfusions, despit corticosteroid treatment.

On examination by the author in *1961* the patient had hypergammaglobulinemia (1.9 g per 100 ml), borderline values of th FII AP-(20) *and FIIA-SC-(64) tests, and a negative test for* ANFs.

Descriptive diagnosis of proband's sister: Transient polyarthritis, drug sensitivity hypothyreosis, tumours of mesenchymal tissues (myoma neurinoma, hypernephroma) *chronic hepatitis* with cirrhosis.

PEDIGREE OF PROBANDS 10 AND 47

Genealogic investigation (Chapter 4) revealed that probands 10 and 47 were *first cousins.* Their common pedigree is shown in Figure 6

The case history of *proband 10* (IV 10 in Figure 6) was given in Chapter 3 (page 50). This 32 year-old woman had a classic type f SLE with severe acute spells (one following abortion) and chronic mani festations in the form of CDLE, arthralgia, and pronounced Raynaud s phenomenon.

The 6_ year-old *father* of the proband (III 3 in Figure 6) had slight hypergam maglobulinemia (1.2 g per 100 ml) and borderline results of FIIA SC test (64) and test f r ANFs (±). He was subjecti l *healthy On an earlier occasion (in* 1! ?) his gammagl bulin le el was 1.3 g per 100 ml, and the SSC test wa positive (! !)

Th 61 y r-old *mother* has had *chronic polyarthr ti* nce the ag f 31 In 19_0 wh n she w h had pericardit nd transient psychosis. In 1959 electrophoresis *showed a gammaglobulin value of 2 4 g* per 100 ml, LE-cell test was negative — On examination by the author in 1961 the gammaglobulin was 1.2 g per 100 ml, the *tests for RFs were negative (on previous* spells in hospital positive SSC-test had sometimes been recorded) as was the test for ANFs.

A 26-year-old *sister* of the proband reported peripheral sensitivity to sunshine and Raynaud s phenomenon. She had a gammaglobulin value of 1 8 g per 100 ml, borderline tests for RFs (SSC 16, FII AP 40 FIIA SC-64), but a negative test for ANFs.

Thus proband 10 had probably got an inherited predisposition for collagen disease both from her father who was subjectively healthy but had serologic abnormalities, and from her mother who had severe polyarthritis. Her sister had physical and serologic abnormalities and above all, pronounced hypergammaglobulinemia, yet she had no clinically detectable SLE.

Proband 47 (IV 9 in Figure 6), who died *at the age of 48 years, had chronic poly* arthritis, possibly Hashimoto s thyroiditis, and SLE nephropathy with "diagnostic" *post mortem* findings. Two of her siblings and 1 of the 2 daughters had slight serologic abnormalities (Figure 6).

PEDIGREE OF PROBAND 14

The case history of the *proband* was described in Chapter 3 (page 40)

The *mother* of the proband was said to have had *chronic polyarthritis* and at the age of 40 years she had developed "nar rowing of the esophagus" and had until death 27 years later been unable to consume solid food The report (which was gi *ven by the proband) could not be verified* since the woman had never been in hospital.

The 30-year-old *daughter* of the proband has *chronic polyarthritis* but at time of study he showed no laboratory abnormal ities.

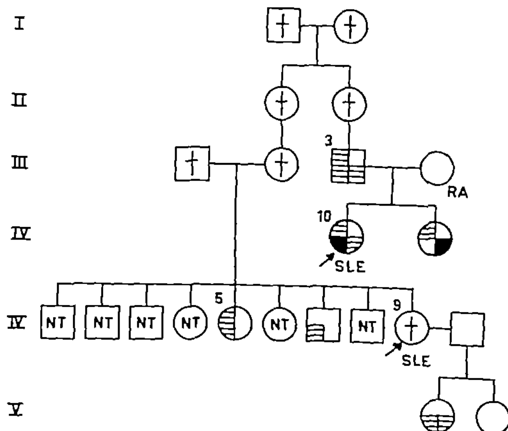


Figure 6. Pedigree of probands 10 and 47

PEDIGREE OF PROBAND 21

The *proband's* history was given in Chapter 3 (page 52). She had had high ESR for several years before th "diagnostic episode of SLE.

The *mother* f the proband, 64 years old, is disabled by *chronic polyarthritis* In 1952 sh had developed strido which proved t be caused by paresis of the laryngeal muscles. Tracheotomy was per formed, and th patient has since lived with a tracheal canulla. On I terview by the uthor in 1062, the patient was found t have an exanthema of *CDLE* type n th f ce Laboratory investigation showed a gammaglobulin value of 1 4 g per 100 ml, strongly positive tests for RFs a positive Meinicke s reaction (TPI test negative), and a + + + positive test for ANFs.

Th 10-year-old *daughter* of the proband had n obscure illness somewhat reminiscent of SLE.

Since the ge of 1 year she had been hospitalized several times because of recurrent infections (enteritis, pharyngitis and titis). I 1958 she had bout of purpura of the legs with swollen ankles, positiv test for ASL (1000 and 500 U), and elevated alpha-2 globulin (0 9) and slightly levated gammaglobulin (1.2 g per 100 ml) levels. The diagnosis was "anaphylactoid purpura In 1961 she had lymphadenitis of the neck, and during treatment with sulfonamides she had a spell f exanthema and the WBC fell to 2500 per cmm. I 1962 the patient had few attacks of sudden loss of consciousness—probably epileptiform ttacks. During all the years the patient was under observation the ESR was never normal—

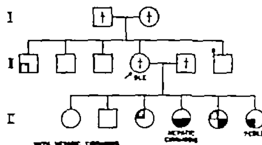


Figure 7 Pedigree of proband 54

micro-ESR usually varied between 20 and 50 mm/1 hour — At laboratory investigation by the author in 1962 the patient was found to have slight hypergammaglobulinemia (1.2 g per 100 ml) but negative test for RF and ANFs.

The father 3 brothers, and the son of the proband showed no serum protein abnormalities.

PEDIGREE OF PROBAND 45

The *proband* had recurrent polyarthritis and chronic nephritis and died at the age of 43 years. Autopsy showed "diagnostic lesions in the kidneys.

The *mother* of the proband died at the age of 68 years in uremia. During the final stage she also had jaundice Autopsy was not performed. The clinical diagnosis was *chronic nephritis* Five years previously she had undergone cholecystectomy Incidentally the WBC was repeatedly low 1100 3100 and 0700

The *father* of the same proband, who was living at time of study is disabled by *chronic polyarthritis*. He had slight hypergammaglobulinemia (1.3 g per 100 ml) borderline tests for RFs (SSC-test negative FII AI 20 FIIA SC- 61) and a + + positive test for ANFs.

A year-old *brother* of the patient had pronounced longstanding arthralgia, but no joint deformities were seen at the time of interview He also had negative reactions for RFs. Another brother 50 years old, had occasionally slight pain in the knees and showed borderline FIIA SC-test (61) and a doubtfully positive test for ANFs.

PEDIGREE OF PROBAND 54

The *proband* (Figure 7) was hospitalized at the age of 60 because of loss of body weight, increasing dyspnea and edema of the legs. Besides signs of cardiac incompensation, investigation revealed profound anemia (Hb 5—7 g per 100 ml), ESR 150 mm/1 hour hypergammaglobulinemia (2.7 g per 100 ml), positive Wassermann and Meinicke tests, roentgenologic signs of previous bilateral pleuritis, and signs of renal damage with microscopic hematuria and increased NPN (61—69 mg per 100 ml). After one week in hospital, the patient suddenly died of an acute myocardial infarction. *Post mortem* showed, besides the acute infarction, aortic and mitral stenosis with histologic signs of previous and present endocarditis of the mitral valves (including suspected Libman-Sacks endocarditis), healed myocarditis, fibrinoid focal necrosis of glomerular tufts, and *early portal cirrhosis of the liver*

The 20-year-old *daughter* of the proband (III 4 in Figure 7) had *chronic hepatitis* with cirrhosis:

In *1959* she developed acute hepatitis with increase of bilirubin to 15 mg per 100 ml, of glutamic pyruvic acid transaminase to 401 U and of gammaglobulin to 3.2 g per 100 ml. She also had bilateral bronchopneumonia. She was treated with corticosteroids and the icterus abated. However one month later she was again jaundiced. Liver biopsy showed acute hepatitis. The spleen was roentgenologically enlarged. The hepatitis gradually became chronic biopsy at the end of 1959 showed "subchronic and at the end of 1960 chronic hepatitis. She had periodically varying degrees of jaundice and elevation of liver enzyme levels: sometimes exacerbations appeared to follow infections of the upper respiratory tract. Since 1961 she has experienced slight arthralgia. Therapeutically the process has been influenced by corticosteroids, and finally a dose could be titered out, on which the patient has felt fairly well with only slight elevation of the bilirubin and transaminase level of the serum. —On examination by the present author in 1962, *the patient had gammaglobulin value of 3.6 g* per 100 ml. She had + positive test for ANFs, but the test for RF were negative

Another daughter (III 6 in Figure 7), 23 years old, on interview in 1962 showed a *longstanding butterfly erythema* of the face she had however not consulted any physician because of this lesion. Laboratory investigation revealed a + positive fluorescence test for ANFs, but the gammaglobulin level was normal and the tests for RFs were negative

OTHER PEDIGREES

Chronic polyarthritis was found also in the 41 year-old sister of proband 16 in the mother (died 78 years old) of proband 19 in the 75-year-old sister of proband 25 in the 74 year-old father of proband 29 and in the 59-year-old sister of proband 49 In addition, the 63-year-old brother of proband 32, living outside Scania, stated that he had chronic polyarthritis, but the information could not be checked.

The mother of proband 22 and the father of proband 53 were said to have died in *chroni nephritis*, but no d tails could be obtained.

A sister of proband 23 died at 44 years in *chronic myeloi leukemia.* During this disease she had had arthralgia and sometimes arthritis.

The 63-year-old father of proband 130 on lectrophoresis for the present study showed a *narrow band*" in the beta-2 region. He also had a positive SSC-test (64) and a borderline FIIA SC-test (32) He was, however subjectively healthy

SLE LIKE SYNDROMES IN RELATIVES B

PEDIGREE OF PROBAND 59

The case report of the *proband* was given in Chapter 3 (page 43). She had CDLE, positive LE-cell phenomenon and a few other manifestations consist nt with SLE.

The *mother* of the proband died *1952* at the age of 62 after prolonged obscure illness. She was said t have had spell f jaundice in *1936* In *1941* and 1942 transient arthralgia and fever During the following years frequent attacks of fever without obvious cause. I *1944*, splenomegaly had been noted for the first time, and during the last years of her ill both the spleen and the liver had been severely enlarged. Since 1944, she had also had varying and sometimes pronounced anemia (RBC 2—3 million per cmm), as well as leucopenia (WBC usually below 4000, often between 2000 and 3000 per cmm) and thrombocytopenia (lowest value 90 000). Presumedly false-positiv STS had been noted on several occasions. She had had signs of renal affection with periodic proteinuria, hematuria and pyuria. *Post mortem* was incomplet \ signs of malignant disease, sarcoidosis or liver cirrhosis were reported. A specimen from the kidney was re-examined (Prof F Linell) and showed chronic pyelonephritis but no signs of SLE.

Descriptive diagnosis f probands mother- Chronic hepato-splenomegaly with hemocytopenia Chronic pyelonephritis.—The case history is somewhat reminiscent of that f proband 14 (page 49).

PEDIGREE OF PROBAND 65

The *proband* a 66-year-old male had chronic polyarthritis, chronic glomerulonephritis and liver cirrhosis, purpura hyper globulinemica and LE-cell phenomenon. But utopsy revealed no signs typical of SLE.

The *mother* of this proband died in 1933, she was then 72 years ld after 4 months illness with hepat megaly jaundice and edema. The jaundice diminished during the course, but the patient died in what was apparently a hepatic coma. The clinical diagnosis was "subchronic hepatitis with cirrhosis but necropsy was not done.

PEDIGREE OF PROBAND 73

The *proband* a 65-year-old female had chronic polyarthritis, bouts of pleuritis, leucopenia considerabl hypergammaglobulinemia, and an abundance of LE-cells in the peripheral blood smear In 1057 the bromsulphalein test showed 26 % retention after 30 minutes. Liver biopsy was not done

The 34-year-old *son* had acute hepatitis in 1950 which progressed into *liver cirrhosis*—the diagnosis was confirmed by biopsy in 1955 In 1960 he began to suffer from diarrhea, and X-ray showed *ulcerative colitis* Of all types of treatment tried, he fared best n corticosteroids The gammaglobulin level varied between 2.7 and 1.2 g per 100 ml (the last value during steroid therapy). LE-cell phenomenon had

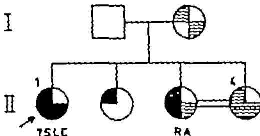


Figure 8. Pedigree of proband 83.

been investigated on 3 occasions with negative results, but at the author's investigation in 1961 the patient had a ++ positive test for ANFs. The gammaglobulin was by then 1.3 g per 100 ml.

Of 4 other sons of the proband studied 1 had a + positive and 1 a ± positive test for ANFs. Both were subjectively healthy. Of 2 daughters studied 1 showed no laboratory abnormalities, while the other, she was 31 years old, had hypergammaglobulinemia (1.5 g per 100 ml). Half a year before the author's investigation she had had a severe "eczema" of the face after exposure to sunshine. The test for ANFs was negative.

PEDIGREE OF PROBAND 83

The *proband*, a 27-year-old female, had Raynaud's phenomenon, recurring arthralgia, widespread discoid LE, spells of fever, striking lymphadenopathy, transient psychosis, transient nephritis, hypergammaglobulinemia and a dubiously positive LE-cell phenomenon. At investigation by the author in 1961 she had no actual complaints but showed hypergammaglobulinemia (2.0 g per 100 ml) strongly positive tests for RFs (SSC- 512, FII AP 640, FIIA SC- 1024) and a + positive test for ANFs.

In a 22-year-old sister of the proband (II 2 in Figure 8) the tests for RFs were borderline or positive (SSC 32, FII AP 80, FIIA SC 16). Another sister, 20 years old (II 3 in Figure 8) had been hospitalized in 1957 and in 1961 because of *rheumatoid arthritis* (positive X-ray, positive SSC-test) and had then also showed slight proteinuria. At investigation by the author in 1961 she still had peripheral symmetric arthritis, gammaglobulin 1.1 g per 100 ml, strongly positive tests for RFs (SSC- 256, FII AP 640 and FIIA SC- 1024) and a ++ positive test for ANFs. Her *monozygotic twin sister* (II 4 in Figure 8) was subjectively healthy but showed the following abnormalities: gammaglobulin 1.1 g per 100 ml, SSC-test 16, FII AP test 20, FIIA SC-test 64 and a ± positive test for ANFs. The diagnosis of zygocity had been established earlier in a twin study by Dencker (1958).

The *mother* (I 2 in Figure 8) of the proband was subjectively healthy but the results of SSC- (32) and FIIA-SC-tests (16) were borderline.

PEDIGREE OF PROBAND 94

The *proband*, a 30-year-old *female*, had a history very *suggestive* of SLE with recurring polyarthritis, epileptiform attacks and a nephrotic syndrome as the most prominent features.

Her 25-year-old *brother* had an obscure disease, namely longstanding *mediastinitis* with obstruction of the superior caval vein. Biopsy in 1960 showed a fibrotic tissue with chronic inflammatory cells, mainly plasma-cells. No signs of specific etiology (such as tuberculosis or syphilis). Gammaglobulin about 1.5 g per 100 ml. Since 1960 he had been on corticosteroids with slight improvement. At the author's examination in 1961 the gammaglobulin value was normal (0.8 g per 100 ml)—but the patient was on corticosteroids—and the only other abnormalities seen were borderline values for SSC and FIIA SC tests (both 16). The biopsy specimens were re-examined in 1963 (Prof C-G Ahlström) but showed no structures consistent with thymoma.—It cannot be excluded that this process represents an unusual form of autoimmune disease.

The 61-year-old *mother* had positive

tests for RFs (SSC-32, FII AP 80 and FIIA SC- 1024) A 37 year-old *sister* of the proband had past polyarthritis but no present abnormalities.

PEDIGREE OF PROBANDS 95 AND 104

Th *proband* a 57 year-old female (II.7 in Figure 9) was described by Krook (1961) as "Case 4 H. D" Signs of systemic disease in the form of swelling of both parotid glands, keratoconjunctivitis sicca, hypergammaglobulinemia and false positive STS had preceded the signs of *liver cirrhosis* by 7 years.—At investigation by the author in 1961 she felt fairly well but had undergone portal-caval anastomosis because of bleeding esophageal varices half a year previously Her gammaglobulin value was now 1 7 g per 100 ml, the Wassermann reaction was positive, as were the tests for RFs (SSC- 128 FII AP 160 FIIA SC- 32) and fo ANFs (+)

A 63-year-old *sister* (II.5 in Figure 9) had had periods f fever high ESR hypergammaglobulinemia and keratoconjunctivitis sicca for 10 years before *liver cirrhosis* was diagnosed. She was described as Case 3, A. P by Krook (1961).—At the present writer's examination in 1961 she had a gammaglobulin value f 2.6 g per 100 ml, strongly positive tests for RFs, a + positive test for ANFs, and positive Wassermann, Meinicke and Kline reactions but negative TPI

A *niece* of proband 95 (III 1 in Figure 9) was included in the proband material as case of *suspected SLE* (proband 104).

This woman, born 1912, sought medical ad ice for angina tonsillaris in *1951*. The ESR was not normalized after the infection and, on repeated examination during 1951—1958 it varied between 20 and 40 mm/1 hour I May *1958*, the patient had fever and arthralgia with swelling f the radiocarpal and talocrural joints She also stated that she had experienced recurrent sw lling of the parotid glands and dryness of mouth and eyes. The SSC-test was positive (128), and electrophoresis showed hypergammaglobulinemia (2.6 g per 100 ml).

The joint symptoms abated, but in April *1959* thrombocytopenic purpura appeared. The thrombocyt counts were down t 10,000 per cmm. The patient also had leucopenia with minimum WBC of 2200 and anemia with minimum Hb of 7.5 g per 100 ml. ESR on dmission 53 mm/1 hour Gammaglobulin 2.2 g per 100 ml. LE-cells were not demonstrable. Ophthalmologist s report: keratoconjunctivitis sicca. The patient was treated with corticosteroids and improved with disappearance of the anemia and thrombocytopenia. During ambulatory follow-up until *1961* no signs of purpura and no thrombocytopenia but persisting increased ESR (20—40 mm/1 hour) despite continual corticosteroid treatment.

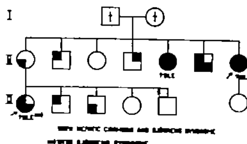


Figure 9 Pedigree of probands 95 and 104.

The *mother* of proband 104 the eldest sister of proband 95 (II.1 in Figure 9) had a + positive result of test for ANFs at the present investigation. This 72 year-old woman had "elevated blood pressure with only minimal subjective complaints. In 1948 she had had toxicodermia with a rash mainly located to the face nd in 1951 apparently the same type of rash which was then called erysipelas *Subacute LE?* She had also had peripheral arthralgia periodically

As is evident from Figure 9 other members of this pedigree also showed serologic abnormalities. Thus, a subjectively healthy 65-year-old *brother* f proband 95 (Figure 9 II 4) showed a *false positive Wassermann reaction* (TPI negative) A 43-year-old *niece* (III 4 in Figure 9) showed no serum protein bnormalities in the present study but she had had erythema exudativum multiforme " in 1943, and in 1950 she had had right-sided pleural ex date with high fever Tuberculosis had then been suspected, but repeated examinations for bacillae had been negative

PEDIGREE OF PROBAND 103

The *proband* a 23-year-old female had a history of recurring pleuritis, exanthema thrombophlebitis, proteinuria and leucopenia. No LE-cells had been found, but at the author's investigation in 1962 the test for ANFs was ++ positive

The *mother* of the proband 45 years old, had had epileptiform (grand mal) attacks f obscure origin since 1055 and since 1056 she had continuously been taking phenemal and phenantoine At the time of the present study (1962) she felt well but was found to have hypergammaglobulinemia (1 1 g per 100 ml) a positive Meinicke test (TPI negative) and a + + + positive test for ANFs.—This woman might have oligosymptomatic SLE or a drug-induced SLE like syndrome

PEDIGREE OF PROBAND 106

The 43-year-old *proband* had periodically had rthralgia since he was about 20 years old. I *1953* she had otitis, and the ESR was 90 mm 1 hour However the levation of the ESR per sl ted—in 1954 lue of 50 mm/1 hour was noted and I 1955 it was 110 mm 1 hour I *1955* she lso had fever and xanthema in connection with salicylazo- alf pyridine treatment for arthralgia, and she later reacted I the same w y t phen ibutazone A gammaglobulin value of 1.9 g per 100 ml was noted, as was fal tly positiv Wassermann reaction and "suspected LE-cells. I *1957* fever bilateral pleuritis with lamellar densities f both lung bases on X-ray peritendinitis, spell of dizziness with yst g mus, hypergammaglobulinemia (2.3 g per 100 ml), positiv W sserman test, and again dubious positive LE-cell phenomenon. The acut course bsided with axillary corticosteroid treatment nd the patient had since done w ll, cept for arthralgia without objective evidence of joint i volvement. I *1961* at present st d the gammaglobulin alue was 1.3 g per 100 ml, test for RF were negativ but test for ANI was – positi

Descripti e diagnosi of proband Chronicall high ESR chronic hypergammaglob linemia, chronic false-positive STS react r t t arthralgia acute episod of SLE?

The 57 year-old *sister* f th proband (II:2 in Figure 10) had had pain in the left leg since 1958 and in the left arm since 1959 thought to be due t spondylosis. The patient had been hospitalized I *1959 1960* and *1961* because of elevated ESR, which was never below 57 mm/1 hour Examinatio had not revealed any cause of the abnormal ESR. Electrophoresis had been performed during each period I hospital and showed gammaglobulin values of 2.5, 2.8 and 2.6 g per 100 ml, respectively In 1961 a WBC of 3300 was recorded and a few typical LE-cells were found in a smear f leucocytes. No subjective complaints gesti of Sjögren syndrome no pathologic taining of the conjunc tivae with Rose Bengal. The bromsulphalein test showed 4 1 % retention after 30 minutes. —When examined by the thor in 1961 the patient had gammaglobulin value of 2.3 g per 100 ml (polyclonal type), a positive FIIA SC-test (256) but negative SSC- and I II AP tests and negative test for ANFs. The Wassermann reaction was faintly positive but the TPI was negative

Descriptive diagnosis of proband's sister Essential" hypergammaglobulinemia. False positive STS reactor

Another *sister* (II 4 in Figure 10), 51 years old, who had never been in hospital, was found to h ve a gammaglobuli value of 2.2 g per 100 ml nd positive SSC-test (64) t the uthor investigation in 1961 Because of the excessive hypergammaglobulinemia he was I estigated in hospital half year later She still f lt well and denied an joint symptoms. Routine physical examination, blood and urine analysis showed nothing abnormal except an elev ted ESR 33 mm/1 hour hypergammaglobulinemia (2.4 g per 100 ml), borderline SSC- (10) and FIIA-SC-tests (32). A LE-cell preparatio was negati as was the fluorescence test for ANFs.

Descriptive diagnosis Essential hypergammaglobulinemia.

A 48-year-old *brother* of the proband (II.5 in Figure 10) had borderline values in all 3 tests for RFs (SSC- 32, I II AP 40 FIIA SC- 64) and a gammaglobulin valu of 1 1 g per 100 ml. At the age of 18 h had had rheumatic fever but had since felt well.

Of the 3 *children* of prob nd 106 (not shown in Figure 10), a 15-year-old son had a borderline FIIA SC test (64).

PEDIGREE OF PROBAND 107

The 49-year-old *proband* had been in hospital in 1946 because of unexplained high ESR (45—35 mm/1 hour) and in 1960 she had had a fairly typical course of SLE with *inter alia* pericarditis and bilateral pleuritis.

Her *mother*, a 75-year-old woman, was found by the author to have a gammaglobulin value of 2.4 g per 100 ml. She was admitted to the Department for Internal Diseases in Malmö for closer examination. She was found to have benign essential hypertension with only very slight symptoms. She denied any joint symptoms or symptoms of Sjögren's syndrome. Routine blood and urine analysis showed nothing abnormal except elevation of the ESR, 66 mm/1 hour (1 year previously a value of 77 mm/1 hour had been recorded in another hospital). The bromsulphalein test showed 7 % retention after 30 minutes. Chest X-ray was normal and X-ray of the hands revealed no signs of arthritis. The gammaglobulin value was now 2.6 g per 100 ml. LE cell test was negative but the tests for RF were strongly positive.—At re-examination 1 year later this woman still had hypergammaglobulinemia (2.1 g per 100 ml) and positive tests for RF but was symptomfree.

Descriptive diagnosis of proband's mother: Essential hypergammaglobulinemia.

The 30-year-old *son* of proband 107 was found to have a *false-positive Wassermann reaction*. When he was 11 he had had a spell of polyarthritis but had been subjectively healthy ever since.

OTHER PEDIGREES

Cases of *chronic polyarthritis* were present also in the pedigrees of proband 72 (a 44-year-old sister), 74 (the 58-year-old mother), 82 (a 36-year-old brother), 87 (an 88-year-old sister), 91 (a 62 year-old sister), 97 (a 43-year-old daughter) and 109 (a 69-year-old sister). Also a sister (dead 28-year-old in tuberculosis) of proband 71 and the mother (dead at the age of 69) of proband 80 were said to have been disabled by chronic polyarthritis, but the information could not be verified by any hospital records. A brother of proband 70 died at the age of 11 in "acute rheumatic polyarthritis" but no details were available.

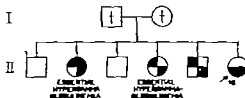


Figure 10 Pedigree of proband 106.

The mother of proband 92 died at the age of 69 after 4 months' illness almost exactly mimicking the above described disease of the mother of proband 65. The clinical diagnosis was *hepatic cirrhosis* but *post mortem* was not performed.

The father of proband 58 died at the age of 68 in *myeloma* which was diagnosed on the basis of typical X ray changes in the skeleton and on the basis of histologic examination of bone-marrow. Also the 55-year-old sister of proband 105 was known to have *myeloma*. She was included in the group of relatives B examined by laboratory tests in the present study. She then showed a monoclonal gammaglobulin increase to 4.8 g per 100 ml. The diagnosis of myeloma had been established previously on the basis of typical X-ray findings and bonemarrow-smears.

A sister of proband 87 had died at the age of 68 in *malignant lymphogranuloma* (Hodgkin's disease)—the diagnosis was verified *post mortem*. Among other manifestations she had had a presumably false-positive STS.

SLE LIKE SYNDROMES IN RELATIVES KG

The *pedigree of proband KG* was described previously by Leonhardt (1957) and by Larsson and Leonhardt (1959). Part of the pedigree is shown in Figure 11.

The *proband* had classic SLE, developing from purpura hyperglobulinemica. She had had butterfly exanthema during the acute courses. The diagnosis of SLE was confirmed *post mortem*.

An elder sister (II.4 in Figure 11) died

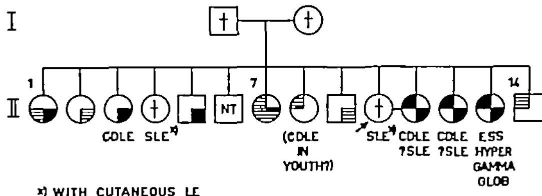


Figure 11 Pedigree of proband KG

in acute severe *SLE* with "diagnostic necropsy findings. She had also had butter fly exanthema

The 44 year-old dizygotic twin of proband KG had recurrent widespread CDLE and serologic abnormalities, of which the most remarkable was massive hypergam maglobulinemia. At re investigation in the present study in 1961 the patient s conditi n had remained essentially benign but hypergammaglobulinemia persisted (.4 g per 100 ml). The test for ANFs was negative but tests for RFs were positive (SSC 32, FII AP 160 FIIA SC- 512). *CDLE or SLE?*

The 41 year-old sister (II 12 in Figure 11) of proband KG had previously been subjectively healthy but she had had an increased ESR since *19 3* (then 43 mm/1 hour) and in 1958 the gammaglobulin was 1 4 g per 100 ml. In April *1960* she de veloped widespread exanthema after ex posure to sunshine She also had slight arthralgia. On examination she showed butterfly exanthema and widespread *CDLE* -diagnosis confirmed by dermatologic consultant and by biopsy She also ha l light peripheral lymphadenopathy and capillary fragility test was positive ESR 30mm 1 hour gammaglobuli 1 8 g per 100 ml LE -cell test negati Blood cell cou ts (incl di g thrombocytes) normal. At the author xamination in Oct ber *1962* the cutaneous lesions had disappeared (without corticosteroid or antimalarial therapy) but hypergammaglobulinemia (1.5 g per 100 ml) persisted. The tests for RFs were SSC- 32, FII AP 40 FIIA SC 256. The fluorescence test for ANFs was negative

The 60-year-old sister (II 3 in Figure 11) developed in 1907 an exanthema of the face which was diagnosed as *CDLE* by a dermatologist. The lesion disappeared after a few months, during which the patient was on antimalarial drugs.

The 49-year-old sister (II.8 in Figure 11) stated that during adolescence she had always had an exanthema of the face in springtime She has since had no cutaneous lesions.

Th 38-year-old sister (II 13 in Figure 11) with *essential" hypergammaglobuli nemia* had remained subjectively healthy and had not experienced arthralgia, Sjögren s symptoms or cutaneous erupti ns. She still (April 196.) had excessive hy pergammaglobulinemia (3.4 g per 100 ml). She had strongly positive tests for RFs (SSC- 64 FII AP 160 and FIIA SC-1024) but th test for ANFs was negative

As described in the preceding hapters hypergammaglobulinemia wa still prevalent in both first and second degree rela tives of proband KG

COMPARISON WITH PUBLISHED SERIES

Several reports are now available on the *familial occurrence of SLE* (Tables 3—5). These reports however deal with isolated families t which attention had been drawn because of the multiple occurrence of SLE.

Holman and Deicher (1960) reported that they had studied the families of 18 patients with SLE. Members of 5 of the families had "symptoms, serological reac tions, and X-ray evidence of rheumatoid arthritis" Among 44 relatives (first and second degree) of 10 patients with SLE Morteo *et al* (1961) found 3 cases of rheu matoid arthritis, 1 case of SLE, and 1 case of arthritis and ulcerative colitis. In their preliminary study in New York City Siegel *et al* (1961) found 7 cases (4 9 %) of rheumatoid arthritis" among 142 first degree relatives of SLE patients against 2 (1 4 %) in 142 matched controls. Der matitis" was found in 11 (%) of the relatives and in 4 (2.8 %) of the controls, eight (5.6 %) relatives had sensitivity to sun or cold against 4 (2.8 %) f the controls. The authors concluded that the frequency of rheumatoid arthritis and va rious nonspecific types of dermatitis appeared to be higher in the SLE group of family members than in the control group Other forms of arthritis and arthralgia, various types of allergy hypertension and rheumatic fever seemed to be comparable in both groups. Other abnormalities were also comparable or too few in number for comparison In a preliminary communication on a family study of SLE in England Ansell and Lawrence (1963) mentioned that "clinical polyarthritis of rheumatoid type" was not more common among 127 relatives of 46 SLE patients than what might be expected in a random population sample.

Hagberg *et al* (1961) described systemic sclerosis in a mother and SLE (with hepatitis) in her daughter

Clinically manifest diseases have also been sought in primary materials of SLE-like syndromes. The familial occurrence of *rheumatoid arthritis* is well-known (see *g* Edström 1941 Lawrence and Ball 1958 and de Blécourt *et al* 1961) More than 50 observations of familial *CDLE* have been published (see *g* Nagy and Darócry 1962). Co-existing systemic sclerosis and dermatomyositis in the same family (mother and son) have been described by Leonhardt (1961), who also reviewed the scanty reports on familial occurrence of these diseases

GENERAL DISCUSSION

SYSTEMIC LUPUS ERYTHEMATOSUS—A SYNDROME

It would appear that the concept of SLE as a well defined disease entity is untenable. The concept of SLE as a *syndrome* with overlapping of other collagen and autoimmune disorders seems to be more realistic (compare Waldenström 1961). The SLE syndrome is composed of a number of more or less characteristic manifestations, but none of these is quite pathognomonic. As yet, there are no generally accepted criteria of SLE, in contrast to what is the case with rheumatoid arthritis (Ropes *et al* 19 9). This state of affairs makes comparisons between different materials of SLE very difficult.

The concept of SLE as a syndrome is based on information from a wide variety of sources.

(1) The study of the natural history of patients with the full-blown picture of SLE shows that many of them have had single manifestations of the syndrome in the form of transient episodes of illness or physical or laboratory abnormalities persisting for months, years or even decades before the "diagnostic episode". This type of development was clearly depicted by *e g* Harvey *et al* (19 4) in their thorough analysis of SLE and was also illustrated by several case histories in the present study (see also Tables 8, 9 and 11).

On the other hand, acute episodes of SLE often merge into chronic oligosymptomatic states (see case reports of probands 5, 10 and 27 in Chapter 3).

(2) SLE often develops from other collagen or autoimmune disorders such as CDLE, rheumatic fever, RA, purpura hyperglobulinemica, "essential" (polyclonal) hypergammaglobulinemia and the false-positive STS reactor state (see Chapters 1 and 3). It may thus be necessary to modify the diagnosis in a given case from time to time according to the variety of manifestations dominating the picture (see also Bencze and Lakatos 1963).

(3) Accordingly, follow up of primary materials of one of the SLE like syndromes such as CDLE or the chronic false-positive STS reactor state shows that some of these patients ultimately develop the full picture of SLE, while others develop intermediate syndromes (Chapter 1) and in a large number the syndrome remains benign.

(4) Clinical analysis of primary materials of the SLE like syndromes shows physical and serologic abnormalities overlapping SLE.

The indistinct limits of SLE do not justify abandonment of this deeply rooted and practical term. Moreover, the overlapping between SLE and other syndromes might imply common etiologic and pathogenetic factors that might be studied from different starting points.

THE ETIOLOGY OF SYSTEMIC LUPUS ERYTHEMATOSUS

SYSTEMIC LUPUS ERYTHEMATOSUS AND AUTOIMMUNITY

As mentioned in Chapter 1, the primary cause of SLE was formerly sought in the connective tissue ("collagen disease"). During the last decade interest has been focussed more and more on immunologic phenomena and SLE has been conceived

as a "multiple autoimmune disorder" or "systemic immunopathy." It is now clearly established that patients with SLE possess an unusual hyperreactivity of the immune system, both against f reign antigens and self antigens. The production of a multitude of antibodies against readily available, immunologically weak antigens (such as nuclear and cyt plasmic constituents) has invoked ideas f SLE as a disturbed tolerance disease (Hijmans *et al* 1961). Hashimoto s disease wo ld represent another type "disturbed antig n disease" where utoimmunization follows the release of antigens normally secluded from the lympho-reticular system and thus not tolerated. However much overlapping exists between SLE and the other collagen and autoimmune disorders regarding the type of immunologic mechanism.

The pathogenetic significance of the autoimmune phenomena is still debatable but antibodies can doubtless produce morbid-anatomic lesions—compare the xperimentally produced nephritis of Masugi (1933) and serum sickness f Dixon (1961) —and clinical symptoms—compare the autoimmune hemolytic anemia, thrombocytopenic purpura and hemophilia. The existence of a cell-bound type of immunologic reaction opens up another approach to the pathogenesis of the autoimmune disorders.

SYSTEMIC LUPUS ERYTHEMATOSUS —A CONSTITUTIONAL DISORDER

The fact that the diagnostic episod f SLE is ften heralded by single symptoms or signs, even for years, has induced many authors t suggest that SLE develops in predisposed persons. Thus Davis and Gutridge (1951) spoke of "a peculiarly sensitive and reactive individual," Haserick (1955) of the "lupus diathesis" and Waldenström (1961) of the "candidate for lupus." The recent attention to the importance of immunologic phenomena has strengthened this concept. Autoimmune disease of SLE type has not been unequivocally produced in experimental animals (see discussion in Immun logical Aspects 1963). On the other hand, an autoimmune disorder with features of SLE regularly develops in a certain inbred strain of mice (Bielschowsky *et al* 1951, Holmes *et al* 1961, Helyer and Howie 1963), which has evidently the appropriate inherited constitution.

PRECIPITATING FACTORS

The course of SLE is often influenced by endo- and exogenous factors, particularly endocrine factors, infections, and physical (sunshine) or chemical agents (see Chapters 1 and 3). It is reasonable t assume that such f ctors might work as triggers for a harmful immunologic mechanism, either by the release of antibodies cross-reacting with the host s own tissues r by the stimulation of forbidden clones directed against self components. The hydralazine syndrome might represent a special instance, where the extrinsic component is of major importance—the syndrome often developing in individuals without apparent predisposition for collagen diseases and then often in males. The relationship between SLE and *drugs* was discussed in Chapters 1 and 3 (see also discussion in Immunological Aspects 1963).

THE GENETICS OF SYSTEMIC LUPUS ERYTHEMATOSUS

FAMILIAL OCCURRENCE OF SLE AND SLE-LIKE SYNDROMES

A fairly larg number of full-length reports n the *familial occurrence of SLE* are now available (Table 4). As classi full-blown SLE is not common (the incidence of newly diagnosed cases being about 10 per million per year see pages 10 and 37), these reports deserve attention and argu for the possibility of hereditary factors. In 3 of the

production of gammaglobulin is controlled by genetic factors (Kulneff *et al* 1955 Wollheim 1961). It might then not be unreasonable to presume a genetic predisposition to *overproduction of gammaglobulin* in some cases. In fact, the data obtained in the present investigation were consistent with the hypothesis that there might exist a "hypergammaglobulinemic" population (Table 40) as opposed to the numerically larger "normogammaglobulinemic population.

One special example of "familial hypergammaglobulinemia" recently gave rise to interesting speculations, namely the so-called *Aleutian disease* in mink (see survey by Wagner 1963) This disease characterized by severe hypergammaglobulinemia and lymphoid infiltration of inner organs, seems to be produced by a particulate agent probably a virus (Henson *et al* 1962). It has been shown that mink homozygous recessive (aa) for the aleutian gene are significantly more susceptible to experimental and to spontaneous aleutian disease than homozygous dominant (AA) or heterozygous (Aa). Although aleutian disease of mink has been regarded as an experimental model for collagen diseases in man, its clinical characteristics and histologic changes do not correspond fully with those found in SLE in man. Attempts to demonstrate LF and rheumatoid factors in the serum of affected animals have proved negative (see discussion by Good in Immunologic Aspects 1963). After all, the most interesting fact about Aleutian disease might be the evidence that genetic factors play a significant role in the quantitative gammaglobulin response to external stimuli.

It is noteworthy that SLE and other collagen and autoimmune disorders have a strong predilection for females, and that females have on the average higher gammaglobulin levels than males (see Figures 3 and 4 compare also Tizzani 1955). This invites speculation about the possibility of the X chromosome being involved in antibody synthesis, a tentative assumption put forward by Burch (1963) in the discussion of the etiology of rheumatoid arthritis. This hypothesis seems to be consistent with observations made in families of patients with congenital agammaglobulinemia. while the patients themselves are usually males, with only one X chromosome some of their female relatives (especially the mothers) having two X chromosomes (perhaps 1 normal and 1 abnormal), show limited defects in the production of immunoglobulins (Heremans 1960 Fudenberg *et al* 1962)

Another observation deserves mentioning in the discussion of gammaglobulin levels, heredity and SLE. Several authors, investigating the serum proteins of both white and non white individuals, state that the latter have on the average higher gammaglobulin levels (see *e g* Pollak *et al* 1961 Fudenberg 1963). While it is very difficult to exclude environmental factors as a cause of these differences, the constancy of this finding even in, for example negroes with a satisfactory standard of living could argue for a real racial, and then probably genetically determined, difference. According to Siegel (1961), SLE is more common in negroes than in whites. It would seem that the gammaglobulin producing system might be more active in negroes, thereby increasing the chances of these individuals having SLE.

The relationship between excessive production of gammaglobulin and the development of SLE gains further support by the observation of chronic hypergammaglobulinemic states sometimes preceding the full SLE syndrome by years: essential" hypergammaglobulinemia, purpura hypergammaglobulinemica (Waldenström) and perhaps also Sjögren s syndrome Such cases might be commoner than widely believed. In the present series many patients with persistent high ESR had been observed for several years before their serum proteins were analysed analysis then showed hypergammaglobulinemia (Chapter 3).

RHEUMATOID FACTORS IN SLE RELATIVES

In the present investigation, clear-cut differences between relatives and controls regarding the appearance of rheumatoid factors were seen nly in the group B relatives. Group A relatives showed nly higher incidences of borderline and positive results of the *FIIA SC-test* than controls.

Also concerning RFs, the reports on SLE families that have as yet appeared do not permit any definite conclusions (see page 93). As is clear from the foregoing discussion (Chapter 8), the incidence of positive tests will depend very much on the structure of the proband material: patients with SLE and chronic polyarthritis having often strongly positive results for RFs, the relatives following the trend of the probands. It is also conceivable that the serum factors causing reactions in the different test systems are not completely identical (see *e g* Winblad 1963).

Several other investigations have how ever revealed a familial tendency to the production of RFs. Relatives of patients with rheumatoid arthritis thus show a higher incidence f RF than control populations (Bremner *et al* 1959 Ball and Lawrence 1961). When the rheumatoid probands were divided in sero-positive and sero-negative, the familial aggregation of positive tests for RFs was confined to the relatives f the sero-positive probands (Ziff *et al* 1958, Ball and Lawrence 1961 De Blécourt *et al* 1962). The same trend was seen in the present material f SLE relatives (A and B pooled) when divided according to the results of SSC-test in the corresponding probands (Table 50).

External stimuli might also be impor tant f the production of RFs. Thus, substances with the physico-chemical and serologic properties of RFs have been produced in laboratory animals by prolonged immunizati n with bacterial antigens (Svartz 1960 Abruzzo and Christian 1961) or with a soluble protein antigen (Abo nd Wager 1961). Chronic infectious diseases in the human, such as leprosy syphilis, tuberculosis, and bacterial endocarditis, are all significantly associated with positive tests for RFs (see survey by Christian 1963). However RFs appearing in such situations seem not to be associated with arthropathy and often disappear after the antigenic stimulation has been withdrawn. It is conceivable that the continued production of RFs in some individuals assumes a special (genetically determined) predisposition and that this is commonly associated with the predisposition for joint disease.

Rheumatoid factors have been conceiv ed as true antibodies, either antibodies against exogenous agents cross-reacting with 7S immunoglobulins, isoantibodies, or autoantibodies against readily available antigens—the 7S immunoglobulins (see survey by Vaughan 1961). Recently fur ther evidence has appeared favouring th antigen-antibody hypothesis, *e g* the specificity of RFs for genetic types of human gammaglobulin (see discussion in Immunologic Aspects 1963). The pathogenetic significance f RF is still debatable, but most authors believe that RF are not directly pathogenetic (see *e g* Franklin 1963). They have instead been conceived as a parallel phenomenon, reflecting an altered immune response of the host (Kellgren and Ball 1959). In any case, the strong relationship between the presence of RFs and joint disease cannot be neglected. There is also some evidence that the presence f positive tests for RFs in pparently healthy individuals at least predisposes to the late development of RA (Ball and Lawrence 1963).

In summary the familial occurrence of RF has been interpreted as indicating an inherited predisposition to produce antibodies to 7S immunoglobulins; the production of RFs reflecting an altered response f the host t certain pathogenetic stimuli. That an inherited abnormal state of the antibody producing system might be the background f familial occurrence of RFs is further supported by the finding of a high incidence of RFs in relatives of pa-

tients with acquired agammaglobulinemia (Fudenberg *et al* 1962).

ANTINUCLEAR FACTORS IN SLE RELATIVES

In the present study the probands rela tives (of group A as well as of group B) showed higher incidences of positive and doubtfully positive results of fluorescence test for ANFs than controls ($P < 0.001$). The few reports hitherto published (page 98) concerning family studies in SLE and including a search for ANFs have given widely varying incidences of positive results, but all investigators agree that ANFs are more common in SLE relatives than in controls. There might thus be a genetic predisposition to the production of these factors.

Antinuclear factors are closely related to SLE—like rheumatoid factors to RA—but are also common in patients with SLE like syndromes. They have been conceived as antibodies against readily available an tigens, namely the nuclear components, and thus as "markers" of disturbed tolerance (see Chapter 1). Their possibly direct pathogenetic effect *in vivo* has been the subject of much debate (Chapter 1) but has not been excluded (the hematoxylin bodies). The presumably abnormal im mune state indicated by ANFs might, however in some or other way predispose to the development of SLE.

FALSE-POSITIVE SEROLOGIC TESTS FOR SYPHILIS IN SLE RELATIVES

False-positive serologic tests for syphilis are a wellknown manifestation of SLE (see Chapter 1) although the incidences vary from series to series (see Table 2). The phenomenon has been ascribed to the appearance of irregular antibodies. Like other serologic abnormalities in SLE, they may be conceived as a manifestation of an altered immune response of the host. It is not known whether the serum factors responsible for the false-positive STS have any pathogenetic significance. They have however often been found to occur together with circulating anticoagul ants (Laurell and Nilsson 1957. Margolius *et al* 1961). False-positive STS may also herald the other manifestations of SLE, sometimes by years (see Chapter 1).

The incidence of false-positive STS in the *relatives* of patients with definite SLE in this series was low—in fact, only 1 instance was found—a woman with chron ic polyarthritis. Among the relatives of patients with suspected SLE there were 5 (2 %) false-positive reactors. Some other authors have reported findings of false-positive STS among SLE relatives (see page 101), but on the whole such cases appear to be relatively uncommon. It is, however noteworthy that the occurrence of false-positive STS might be confined to certain families (see Chapter 10) so that in these instances it might be a question of a hereditary predisposition. In this connection it is of interest to note that a false-positive STS (negative TPI) has been described in 4 apparently healthy female members (mother + 3 daughters) of the same family (Cannon 1958).

ANTISTREPTOLYSIN IN SLE RELATIVES

As discussed above, there was evidence in the present series that some SLE patients are prone to produce high titres of antistreptolysin. The information obtained in the literature is equivocal (see page 13).

Investigation of ASL in *relatives* of pa tients with SLE have not been reported by other authors. The results of ASL deter minations in the present study were difficult to interpret. The frequency of border line and and positive results of ASL-test was significantly higher among *female* relatives A than among female controls. The frequency of such values among the *male* relatives A was, if anything, lower than that among the male controls, but the difference was not significant. The number of borderline and positive tests was equally

common among the female relatives B as among female controls; the frequency was significantly lower for the male relatives B than for the male controls. The inconsistency of the results might be attributed to the fact that the ASL titres are strongly influenced by environmental factors—*i.e.* the frequency of streptococci, which is different in different seasons of the year *etc*—factors which are not easily controlled in an investigation like this. The incidence of positive tests among the present control series was thus much higher than in the control materials collected by Ingestad and Winblad (1963). It cannot be excluded, however, that the markedly high incidence of positive tests among female relatives A might reflect the same trend found for the corresponding probands and thus might possibly be dependent upon an unusual hyper reactivity of the antibody forming system. Such hyper-reactivity also distinguished pedigree KG and might thus be more important in some families than in others.

THE SPECTRUM OF ABNORMALITIES IN SLE RELATIVES

Many of the physical and serologic abnormalities studied were found to be interrelated, often occurring together in the same individuals (see Chapter 12). These abnormalities were pooled in an attempt to bring out the differences between SLE relatives and controls still more clearly. It was then seen that high scores for abnormalities relevant to SLE were much commoner in the relatives than in the controls (page 109).

Some of the abnormalities studied sometimes occur as *early* manifestations of SLE, *e.g.* arthralgia, Raynaud phenomenon, hypergammaglobulinemia and false-positive STS. Other abnormalities—ANFs, RFs—might conceivably also be early signs of the process leading to SLE. The co-occurrence of multiple abnormalities in the same individual might then be still more ominous—compare the gradual accumulation of symptoms and signs in some SLE patients.

DEFINITE AND SUSPECTED SYSTEMIC LUPUS ERYTHEMATOSUS

Owing to the difficulties to define SLE, two groups of probands were studied for genetic factors. One group (A) consisted of patients with at least 1 "diagnostic" criterion in the form of cutaneous LE, LE cell phenomenon or typical histologic lesions *post mortem*. In addition, all the patients had a clinical picture approaching the classic "text-book" descriptions of SLE. The other group of probands (B) consisted of patients with 1 "diagnostic" manifestation but with somewhat atypical history, patients without "diagnostic" manifestations, and patients with SLE-like syndromes (CDLE, RA, Sjögren's syndrome, chronic hepatitis, purpura hyperglobulinemica) and overlapping features.

Regarding physical and laboratory abnormalities, the two groups showed quantitative, rather than qualitative differences. Owing to selection, manifestations such as chronic polyarthritis, Sjögren's syndrome and chronic hepatitis were more common among probands B but were represented also among probands A. Regarding the laboratory variables specially studied (Chapters 6—12), the differences were also quantitative more than qualitative. Thus, both groups showed significantly (t) higher incidences of hypergammaglobulinemia, borderline and positive results of test for RFs and ANFs, and false positive STS, than the controls. When compared with each other the two groups of probands differed significantly (to) only regarding the tests for RFs, which were more often positive among probands B, and regarding ASL, borderline and positive titres being more prevalent among probands A.

Also the *relatives* of the two groups of probands showed only limited differences. Both groups of relatives had higher inci-

dences (to) of hypergammaglobulinemia, borderline and positive results of the FIIA SC test and of the test for ANFs than the controls. Relatives B were also more often positive in the SSC- and FII AP tests than the controls, female controls A had a more pronounced tendency to produce ASL than the controls. Five relatives B and 1 of group A relatives had false-positive STS, which was not encountered in any of the controls. On comparison between the groups of relatives, on the other hand no significant differences were found concerning the RFs, although the relatives B tended to show positive SSC-results more often than the relatives A—compare the findings in the corresponding probands. The relatives A had a tendency to higher positivity in the test for ANFs—like the corresponding probands—and female relatives A were more often positive in the test for ASL than relatives B. The results are in agreement with the concept of SLE as an ill-defined syndrome the features dominating the picture varying from case to case.

Objections against the results described could be risen owing to the fact that the collection of probands and of relatives was not complete (Chapters 2 and 3). This might have caused bias, as SLE patients with a strong hereditary predisposition would conceivably attract more attention and be more easily diagnosed than patients without such predisposition. However the collection of probands with *definite* SLE diagnosed in Malmö was probably nearly complete (page 37). The incidences of various physical and laboratory abnormalities in the relatives of those probands (totally 62 individuals) appeared in no way to differ from the incidences in the relatives of probands selected from hospitals outside Malmö (totally 163 individuals). Thus, when the former were compared with the latter regarding the incidence of respondents with abnormalities scoring 2 + or more (page 109) the difference between observed and expected numbers was + 4 2 and the mean error 3 1 — no statistically significant difference. Neither was there any difference between the relatives of Malmö probands with *suspected* SLE (total number 109) and the relatives of probands from other hospitals (total number 108) in this respect—the corresponding figures being —0 9 (difference) and 3 2 (mean error).

MODE OF INHERITANCE

As discussed above there is strong evidence in favour of the importance of genetic factors in the etiology of SLE. Also the study of Lilshay and Siegel (1963) on the birth order and the occurrence of SLE is consistent with the concept of genetic mechanisms in the causation of SLE. There seems however to be no simple mode of inheritance. Even in the descriptions of "classic" SLE occurring in families, the clinical pictures never overlap completely On the contrary there is a wide range of physical and laboratory abnormalities, of obscure illnesses reminiscent of SLE and of clinically recognizable SLE-like syndromes in the relatives of SLE patients. In some families, one or another "specific" feature is predominant and reflects the underlying abnormality—compare the hypergammaglobulinemia and the cutaneous LE manifestations in pedigree KG—but in most families there is a mixture of various laboratory and clinical abnormalities. Most of these can however be covered by the blanket term "autoimmunity"

It is striking that all physical and laboratory abnormalities studied in the SLE patients and in their relatives occur also in controls. Thus hypergammaglobulinemia RFs, and occasionally ANFs can be produced also by apparently healthy individuals. But they are produced in a higher incidence in the SLE relatives and usually also in larger quantities. Not only the SLE patients but also their relatives thus show an unusual immunologic responsiveness. Hypothetically the primary cause might then be a genetically determined weakness of the whole immune system—

expressing itself in many different ways (as physical or serologic abnormalities) depending on environmental conditions. Thus, infections, physical and chemical agents might in the predisposed individuals release a self-perpetuating production of injurious autoantibodies, sometimes leading to clinically apparent disease (especially in women).

The underlying genetic factors might be multiple. This would be in analogy with what has been proposed by Lawrence *et al* (1961) for RA, namely that one factor might favour the appearance of clinical arthritis and one or more factors might be responsibl for the production of RFs. As for SLE, in some cases the tendency to overproduction of (normal and abnormal) gammaglobulin (a gene for hypergammaglobulinemia?) might be the most important inherited mechanism, genes for arthritis and for the production of RFs might sometimes modify the clinical picture, an inherited weakness of organs such as the skin, the salivary glands, or the liver might make these organs targets for autoimmune processes in certain pedigrees.

In the approach to the etiology of SLF and related syndromes it would appear wise to adopt a dynamic point of view *i e* focus interest on the mechanisms involved in the individual cases rather than on the external signs of more or less distinguishable syndromes. Elucidation of these mechanisms, their pathogenetic importance and their environmental or genetic background might result in a better understanding of the collagen and autoimmune syndromes, of which SLE forms a part.

SUMMARY

The aim of the present study was to elucidate the significance of genetic factors in SLE.

The patients with SLE to be investigated for such factors (the *probands*) were selected from a primary material consisting of 328 patients cared for in hospitals in Scania during the years 19.5—1961 and in whom SLE had been suspected because of certain symptoms and/or clinical findings, including cutaneous LE-cell phenomenon and/or characteristic *post mortem* findings. Of these 328 patients, 218 were excluded because they had ultimately been found to have other unrelated diseases, because they had only minor systemic involvement consistent with SLE, or because they had been inadequately investigated.

Owing to the diffuseness of the concept of SLE and lack of generally accepted diagnostic criteria in the literature, the probands were classified as having "definite or as having suspected SLE according to the firmness of the diagnosis. Those with 2 "diagnostic" criteria (cutaneous LE, positive LE-cell phenomenon and characteristic *post mortem* findings) *and those with 1 "diagnostic" criterion* and a case history consistent with SLE were assigned to the "definite" category (proband group A). This group comprised 57 probands and group B 52 probands. The probands with 1 diagnostic criterion but less typical history those without diagnostic criteria and several cases of SLE-lik syndromes (chronic polyarthritis, Sjögren s syndrome chronic hepatitis etc) with features overlapping SLE were called suspected (probands B) One proband with definite SLE (proband KG) was reported separately as being the case which had initiated the present study

The 2 groups of probands were compared with 5 large literature series of SLE patients. No definite differences were found between group A and the literature series regarding types and incidences of manifestations, while in group B owing to selection systemic involvement tended to be less pronounced.

Clinical analysis of the probands revealed such manifestations as CDLE, chronic polyarthritis, purpura hyperglobulinemica and chronic hepatitis in both groups. Such manifestations often preceded the diagnostic episode of SLE by years. The sometimes gliding transitions between the SLE like syndromes and full-blown SLE were stressed.

Forty-six probands A and 45 probands B still living in 1900—1963 were studied with laboratory analysis, including blood grouping according to the ABO system, electrophoresis of serum proteins tests for RFs, fluorescence test for ANFs, STS, and test for ASL. On comparison between *the groups of probands regarding the* results of the various tests, significant differences were found only for the RFs, positive results being more prevalent among probands B ($P < 0.01$ to $P < 0.05$) than among probands A, and for ASL, borderline and positive titres being more prevalent among probands A ($P < 0.05$). The higher incidences of positive tests for RFs among probands B were due to the fact that chronic polyarthritis, Sjögren s

syndrome and chronic hepatitis were more common among these probands.

Two hundred and twenty five first degree *relatives* of probands A and 217 of probands B living in Scania during the years 1960—1963, were selected for studies by interview and by laboratory analysis.

There were no significant differences between the groups of relatives concerning physical abnormalities, blood groups, gammaglobulin levels, results f tests for ANFs and for ASL. Concerning RFs, the relatives B showed a higher incidence of posi tive results of th SSC test but not of the FII-AP or FIIA SC-tests, the latter representing another type of reactant (human gammaglobulin) than the f rmer (rabbit gammaglobulin) and thus possibly not demonstrating quite the same serum protein factors. Relatives A had a tendency to higher positivity in the test for ANFs, and the test for ASL was more often positive among female relatives A than among femal relatives B. It was concluded that the differences between the groups of relatives were quantitative rather than qualitative and depend nt on the corresponding trends among th probands.

The spouses of the probands and of the probands relatives living in Scania at the time of study served as *controls*. Accord ing to the classification of the probands and probands relatives there were 2 groups f controls, A and B (spouses of proband KG and relatives KG included in group A). It is probabl that the 2 groups represented partly different environmental conditions. However th distributions of the physical and laboratory variables (with the exception of the blood groups) studied did not differ significantly between the groups f controls, which were therefore pooled t btain a larger control material.

Peripheral arthralgia, sensitivity to cold and possibly also sensitivity t unshine and drug sensitivity were more common among relatives f group A as well as of group B than am g th controls.

R latives A and relatives B had significantly ($P < 0.01$ and < 0.001 respectively) higher gammaglobulin levels than the controls. The incidences of doubtfully positive and positive results of the test for ANFs were also significantly ($P < 0.001$) higher in both groups of relatives than in the controls. Relatives B had significantly ($P < 0.01$ and < 0.05, respectively) higher incidences of borderline and positive results of SSC- and FIIA SC-tests than the controls, whil the differences regarding the FII AP-test were not quite significant ($P = 0.05—0.10$). Relatives A had higher incidences of borderline and positive FIIA SC-results than the controls ($P < 0.05$), while the differences regarding the FII AP-test were not quite significant ($P = 0.05—0.10$), and the results of the SSC-test were similar A few cases of false-positive STS were found in the groups of relatives but not in th controls. Finally female relatives A had higher incidences of borderline and positive titres of ASL than the female controls ($P < 0.001$).

When the respondents were given a score based on the physical and laboratory abnormalities relevant to SLE, the difference between relatives and controls was very clear—the f rmer having significantly ($P < 0.001$) higher incidences f 2 + or higher scores.

An account was given f the presence f SLE like syndromes in the families studied (Chapter 13). This account incl ded information concerning relatives living utsid the study area and deceased relatives. Such syndromes were common and varied from obscure illness somewhat reminiscent of SLE to the fully developed picture of SLE.

The pedigree KG (includi g siblings, nephews and nieces f proband KG) was distinguished by high incidences f cutaneous LE, hypergammaglobulinemia and positive ASL titres, while th incidence of reactions in the test for ANFs was low Th whole spectrum of transitions between CDLE and SLE was represented among the sibling of the proband.

The *conclusions* drawn from the tudies

were that SLE is not a distinct disease entity but rather a syndrome closely related to other collagen and autoimmune diseases that there is a wide range of physical and laboratory abnormalities relevant to SLE in the relatives of patients with this syndrome; that such abnormalities are on the whole more common among SLE relatives than among controls; that this familial aggregation of abnormalities argues for predisposing genetic factors.

The tendency to hypergammaglobulinemia and the occurrence of RFs and of ANFs in the SLE relatives were interpreted as markers of an inherited abnormality of the immunologic system. Thus hypergammaglobulinemia might mean a quantitatively abnormal response to endogenous and exogenous stimuli, and the RFs and ANFs—commonly conceived as autoantibodies—might be the markers of "disturbed tolerance." The SLE and SLE like syndromes might be conceived as a consequence of the abnormal immune response, other genetic or environmental factors possibly modifying their appearance.

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APPENDIX

Appendix Table I (cont.)

Proband number	26	27	28	29	30	31	32	33	34	35
Sex	F	F	F	F	F	F	M	F	F	M
Age at onset	34	19	54	30	27	22	59	60	17	53
Age at diagnosis	42	28	60	38	49	35	63	63	32	55
Age at study/death	45	33	66	39	53	37	66	65	38	57
Remittent course	+	++	++	++	++	++	++	++	++	+
Fever	0	++	++	++	++	+	++	++	++	++
Cutaneous LE	D	(+)	0	0	0	0	0	0	0	0
Purpura	0	0	0	0	0	0	0	+	0	0
Other exanthems	0	+	+	+	+	+	+	+	+	0
Loss of hair	0	+	0	0	0	0	0	+	0	0
Arthropathy	+	++	++	++	++	++	0	++	++	++
Lymphadenopathy	0	+	+	+	+	+	+	0	+	(+)
Splenomegaly	0	0	+	+	0	0	+	0	+	+
Hepatomegaly	0	0	+	(+)	+	0	(+)	0	0	+
Pulmonary lesions	0	+	+	0	+	+	+	+	+	+
Pleuritis	0	++	+	+	++	+	++	++	+	++
Myocarditis	0	++	0	(+)	0	+	(+)	0	++	0
Pericarditis	0	0	++	++	0	++	0	++	0	++
Raynaud' phenomenon	+	+	(+)	0	+	+	0	0	0	+
Phlebitis, leg ulcers	+	+	0	0	+	0	0	+	+	0
Abdominal crisis	0	0	0	0	0	0	0	+	+	0
Hepatitis	(+)	0	0	(+)	(+)	0	(+)	(+)	0	(+)
Sialadenitis	0	0	0	+	0	0	0	+	0	0
Keratoconj sicca	0	0	0	0	0	0	0	0	0	0
Iritis, retinitis	0	0	0	0	+	+	0	0	0	0
CNS lesions	0	(+)	—	0	+	0	0	0	(+)	0
Nephropathy	—	+	+	—	—	+	++	—	—	—
Anemia	—	+	++	++	(+)	++	+	—	+	(+)
Leucopenia	+	++	—	++	++	+	++	—	—	++
Thrombocytopenia	+	++	0	+	0	—	++	—	—	—
Hypergammaglobulinemia	++	++	++	++	++	++	++	++	++	++
LE-cell phenomenon	D	D	D	D	D	D	D	D	D	D
STS, false-positive	—	—	++	—	(+)	(+)	—	(+)	(+)	—
Post mortem finding.										
Total number D	2	1	1	1	1	1	1	1	1	1
Total number +	8	24	21	20	20	20	19	19	18	17
Total number (+)	1	2	1	3	3	1	3	2	2	3

Appendix Table I (cont.)

36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
F	F	F	F	F	F	F	F	M	F	F	F	F	F	F
36	44	29	28	32	50	20	58	39	33	17	29	35	21	22
37	44	40	34	32	52	20	64	40	43	39	48	48	22	33
37	51	40	36	53	55	25	74†	46	43†	46	48†	53	26	34
+	++	+	++	++	++	+	++	+	+	+	+	++	+	++
++	++	+	++	++	++	(+)	+	+	++	+	(+)	++	+	++
(+)	D	(+)	D	D	0	0	D	0	0	D	(+)	(+)	D	0
0	0	0	0	0	0	+	0	+	0	0	0	0	0	0
+	0	+	+	+	0	0	0	+	+	+	+	+	0	+
+	0	+	+	+	0	0	0	0	0	0	0	+	0	0
++	++	++	++	++	++	++	(+)	++	++	++	++	++	+	++
+	+	+	0	0	0	+	0	0	+	+	+	0	+	0
0	0	0	0	0	0	+	+	0	0	0	0	0	0	0
0	0	0	0	0	0	(+)	+	0	0	0	0	(+)	0	0
0	+	+	0	0	+	0	+	+	0	0	+	+	(+)	0
+	+	+	0	+	++	0	+	0	0	0	0	+	++	+
+	(+)	+	0	0	+	(+)	0	0	0	(+)	0	0	0	+
0	0	0	0	0	0	++	0	0	0	0	0	0	0	++
0	0	0	0	+	0	0	0	0	0	+	0	+	0	0
0	+	0	+	+	0	0	0	0	+	0	+	0	(+)	0
0	0	0	+	0	0	0	0	0	0	0	0	0	0	0
(+)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	+	0	0	0	0	0	0	0	(+)	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
—	+	+	+	+	—	—	+	++	++	+	++	—	—	—
+	(+)	+	—	(+)	—	++	+	+	(+)	—	—	(+)	++	(+)
++	++	++	++	++	++	—	++	++	+	+	+	+	+	—
—	+	0	0	—	+	++	—	—	—	++	—	—	0	0
++	++	++	++	+	++	++	++	++	++	++	++	0	+	+
D	—	D	—	—	D	D	0	D	—	0	—	D	—	D
—	—	—	—	—	—	—	+	—	(+)	—	(+)	(+)	++	—
									D		D			
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	16	16	16	15	15	14	14	14	13	13	12	12	12	12
2	2	1	0	1	0	3	1	0	2	1	4	4	2	1

Appendix Table I (cont)

Proband number	26	27	28	29	30	31	32	33	34	35
Sex	F	F	F	F	F	F	M	F	F	M
Age at onset	34	19	54	30	27	22	59	60	17	53
Age at diagnosis	42	26	60	38	49	35	63	63	32	55
Age at study/death	45	33	66	39	53	37	66	65	38	57
Remittent course	+	++	++	++	++	++	++	++	++	+
Fever	0	++	++	++	++	+	++	++	++	++
Cutaneous LE	D	(+)	0	0	0	0	0	0	0	0
Purpura	0	0	0	0	0	0	0	+	0	0
Other exanthema	0	+	+	+	+	+	+	+	+	0
Loss of hair	0	+	0	0	0	0	0	+	0	0
Arthropathy	+	++	++	++	++	++	0	++	++	++
Lymphadenopathy	0	+	+	+	+	+	+	0	+	(+)
Splenomegaly	0	0	+	+	0	0	+	0	+	+
Hepatomegaly	0	0	—	(+)	+	0	(+)	0	0	+
Pulmonary lesions	0	+	+	0	+	+	+	+	+	+
Pleuritis	0	++	+	+	++	+	++	++	+	++
Myocarditis	0	++	0	(+)	0	+	(+)	0	++	0
Pericarditis	0	0	++	++	0	++	0	++	0	++
Raynaud' phenomenon	+	+	(+)	0	+	+	0	0	0	+
Phlebitis, leg ulcers	+	+	0	0	+	0	0	+	+	0
Abdominal crisis	0	0	0	0	0	0	0	+	+	0
Hepatitis	(+)	0	0	(+)	(+)	0	(+)	(+)	0	(+)
Sialadenitis	0	0	0	+	0	0	0	+	0	0
Keratoconj sicca	0	0	0	0	0	0	0	0	0	0
Iritis, retinitis	0	0	0	0	+	+	0	0	0	0
CNS lesions	0	(+)	—	0	+	0	0	0	(+)	0
Nephropathy	—	+	+	—	—	+	++	—	—	—
Anemia	—	+	++	++	(+)	++	+	—	+	(+)
Leucopenia	+	++	—	++	++	+	++	—	—	++
Thrombocytopenia	+	++	0	+	0	—	++	—	—	—
Hypergammaglobulinemia	++	++	++	++	++	++	++	++	++	++
LE-cell phenomenon	D	D	D	D	D	D	D	D	D	D
STS, false-positive	—	—	++	—	(+)	(+)	—	(+)	(+)	—
Post mortem finding.										
Total number D	2	1	1	1	1	1	1	1	1	1
Total number +	8	24	21	20	20	20	19	19	18	17
Total number (+)	1	2	1	3	3	1	3	2	2	3

Appendix Table I (cont.)

86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
F	F	F	F	F	M	F	F	F	F	F	F	F	F	F
63	70	7	24	52	61	56	41	17	37	41	58	36	54	10
63	70	10	41	53	61	64	53	29	52	48	68	45	55	47
63†	73	12†	45	56	63	71	61	30	5	49	71†	46†	55†	52
(+)	(+)	+	+	(+)	(+)	+	++	++	++	+	++	++	−	+
++	+	+	++	0	++	+	++	+	+	+	+	++	++	+
0	0	D	(+)	(+)	0	0	(+)	0	0	(+)	0	(+)	0	(−)
0	0	0	0	0	0	0	+	0	0	0	+	0	0	+
0	0	0	+	0	0	+	0	0	0	+	0	+	+	0
0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
++	++	++	++	−	++	++	+	++	+	++	++	++	++	++
0	0	(+)	0	0	0	+	0	+	+	0	0	0	0	0
0	0	0	0	0	0	0	0	+	+	+	0	+	0	+
0	0	0	0	0	0	0	0	+	+	0	(+)	0	0	+
0	+	0	0	0	0	+	+	0	+	+	+	+	+	0
+	+	0	0	0	+	++	++	++	0	0	++	++	++	0
0	(+)	+	0	0	0	0	++	0	0	0	(+)	++	++	0
0	0	0	0	0	0	+−	0	++	0	0	0	0	++	++
0	0	+	0	0	0	0	0	0	0	+	0	0	0	0
+	0	0	0	0	0	0	+	0	0	0	+	0	0	0
0	0	+	0	0	0	0	0	0	0	0	0	+	0	0
0	0	0	0	0	(+)	0	0	0	+	0	0	0	0	0
0	0	0	0	(+)	0	+	0	0	+	0	+	0	0	0
0	0	0	0	+	0	+	0	0	+	0	+	0	0	+
0	0	0	0	0	0	0	0	+	0	0	0	0	0	0
0	(+)	0	0	0	0	0	0	+	0	0	0	0	0	0
−	(+)	−	−	−	−	−	(+)	++	−	−	−	+	(+)	−
+	−	−	+	−	−	+	+	−	+	+	(+)	−	−	(+)
−	−	−	−	+	−	+	−	−	−	++	+	−	−	++
−	0	0	0	0	0	++	++	0	+	++	0	0	0	−
+	++	0	0	++	−	++	++	−	−+	++	++	0	+	++
D	D	−	D	D	D	(+)	−	(+)	−	(−)	−	−	−	−
−	−	−	−	−	−	−	−	−	++	−	(+)	−	−	−
											(+)	(+)	(+)	
1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
8	7	7	7	8	8	19	18	18	17	16	15	15	15	14
1	4	1	1	3	2	1	2	1	0	2	5	2	2	2

Appendix Table I (cont.)

Proband number	101	102	103	104	105	106	107	108	109	KG
Sex	F	F	F	F	F	F	F	F	F	F
Age at onset	30	27	18	39	37	21	47	36	57	22
Age at diagnosis	41	31	18	47	43	38	48	66	62	36
Age at study/death	46	33	23	50	47	43	49	75	63	39†
Remittent course	++	+	+	+	+	+	(+)	++	+	++
Fever	+	+	+	+	0	+	++	+	++	++
Cutaneous LE	0	0	0	0	(+)	(+)	0	0	0	D
Purpura	0	0	0	+	0	0	0	0	0	+
Other exanthema	+	0	+	0	0	+	0	+	0	+
Loss of hair	0	0	0	0	0	0	0	0	0	0
Arthropathy	++	++	0	++	0	++	++	++	0	++
Lymphadenopathy	+	+	0	0	0	0	0	0	(+)	+
Splenomegaly	0	0	0	0	0	0	0	0	0	0
Hepatomegaly	0	0	0	0	0	0	0	0	0	0
Pulmonary lesions	0	+	+	0	0	+	+	+	+	+
Pleuritis	+	++	++	0	+	+	+	0	+	++
Myocarditis	0	0	0	0	0	0	+	0	0	0
Pericarditis	++	0	0	0	0	0	++	0	0	0
Raynaud's phenomenon	+	0	0	0	0	+	0	0	0	0
Phlebitis, leg ulcers	0	0	+	0	0	0	(+)	0	0	0
Abdominal crisis	0	0	+	0	0	0	0	0	0	0
Hepatitis	0	0	0	0	0	0	0	(+)	0	0
Sialadenitis	0	0	0	+	0	0	0	+	+	0
Keratoconj sicca	0	0	0	+	+	0	0	+	+	0
Iritis, retinitis	0	0	0	0	0	0	0	0	0	0
CNS	0	0	0	0	0	0	0	0	0	0
Nephropathy	—	++	+	—	—	—	—	—	—	++
Anemia	—	(+)	+	+	+	—	—	—	+	+
Leucopenia	+	+	+	+	++	—	—	—	—	++
Thrombocytopenia	—	0	—	++	++	0	0	—	—	++
Hypergammaglobulinemia	++	+	++	++	++	++	++	++	++	++
LE-cell phenomenon	—	(+)	—	—	—	(+)	—	0	—	D
STS, false-positive	—	+	—	—	++	+	—	—	—	—
Post mortem findings										D
Total number D	0	0	0	0	0	0	0	0	0	3
Total number +	14	13	13	13	12	11	11	11	10	21
Total number (+)	0	2	0	0	1	2	2	1	1	0

Appendix Table II. Distribution, among titres, of SSC-test in probands, probands relatives and controls

Group	Sex	Age	T tal tested	Titres of SSC-test								
				0	8	16	32	64	128	256	512	1024
Probands A	Males	<45	0	0	0	0	0	0	0	0	0	0
		≥45	4	1	0	0	2	1	0	0	0	0
	Females	<45	18	10	0	3	2	3	0	0	0	0
		≥45	15	11	0	1	1	0	1	0	0	1
Probands B	Males	<45	1	0	0	0	0	0	1	0	0	0
		≥45	1	1	0	0	0	0	0	0	0	0
	Females	<45	12	4	0	0	1	3	1	1	1	1
		≥45	21	7	0	2	2	2	3	3	1	1
Relatives A	Males	<45	62	50	0	8	1	2	1	0	0	0
		≥45	53	46	0	4	0	3	0	0	0	0
	Females	<45	57	50	0	6	1	0	0	0	0	0
		≥45	53	45	0	3	3	1	0	1	0	0
Relatives B	Males	<45	56	44	0	10	0	0	1	0	0	1
		≥45	55	42	0	7	4	1	1	0	0	0
	Females	<45	59	40	0	10	4	2	1	1	1	0
		≥45	46	35	0	3	4	1	3	0	0	0
Controls A	Males	<45	48	44	0	2	0	1	1	0	0	0
		≥45	51	46	0	2	1	1	0	1	0	0
	Females	<45	55	50	0	4	1	0	0	0	0	0
		≥45	30	26	0	0	2	1	1	0	0	0
Controls B	Males	<45	36	29	0	6	1	0	0	0	0	0
		≥45	44	36	4	0	2	2	0	0	0	0
	Females	<45	22	20	0	0	1	1	0	0	0	0
		≥45	37	29	0	6	1	1	0	0	0	0

Appendix Table III. Distribution, among titres, of FII AP-test in probands, probands relatives and controls

Group	Sex	Age	Total tested	Titres of FII AP-test										
				0	10	20	40	80	160	320	640	1280	2560	5120
Probands A	Males	<45	0	0	0	0	0	0	0	0	0	0	0	0
		≥ 45	3	1	0	0	0	2	0	0	0	0	0	0
	Females	<45	18	12	0	1	4	0	0	1	0	0	0	0
		≥ 45	16	12	0	1	1	1	0	0	0	0	0	1
Probands B	Males	<45	1	0	0	0	0	0	1	0	0	0	0	0
		≥ 45	1	1	0	0	0	0	0	0	0	0	0	0
	Females	<45	12	4	0	0	2	1	2	1	1	0	1	0
		≥ 45	21	5	0	1	3	2	4	3	0	1	2	0
Relatives A	Males	<45	62	60	0	1	1	0	0	0	0	0	0	0
		≥ 45	53	50	0	2	1	0	0	0	0	0	0	0
	Females	<45	57	54	1	0	1	1	0	0	0	0	0	0
		≥ 45	53	44	0	2	5	1	0	0	1	0	0	0
Relatives B	Males	<45	56	53	0	1	1	0	0	0	0	0	1	0
		≥ 45	55	50	0	0	3	1	1	0	0	0	0	0
	Females	<45	60	53	0	1	1	1	2	0	2	0	0	0
		≥ 45	46	40	0	1	1	1	0	1	1	1	0	0
Controls A	Males	<45	49	46	0	1	2	0	0	0	0	0	0	0
		≥ 45	51	48	0	0	1	0	1	1	0	0	0	0
	Females	<45	55	55	0	0	0	0	0	0	0	0	0	0
		≥ 45	30	30	0	0	0	0	0	0	0	0	0	0
Controls B	Males	<45	36	36	0	0	0	0	0	0	0	0	0	0
		≥ 45	44	40	0	0	2	0	2	0	0	0	0	0
	Females	<45	22	20	1	0	1	0	0	0	0	0	0	0
		≥ 45	37	34	0	0	0	1	1	1	0	0	0	0

Appendix Table IV Distribution, among titres, of FIIA SC-test in probands, probands' relatives and controls

Group	Sex	Age	Total tested	Titres of FIIA SC-test								
				0	8	16	32	64	128	256	512	1024
Probands A	Males	<45	0	0	0	0	0	0	0	0	0	0
		≥45	3	0	0	1	0	0	0	1	0	1
	Females	<45	18	8	0	5	1	1	1	0	1	1
		≥45	16	9	0	1	3	0	1	0	1	1
Probands B	Males	<45	1	0	0	0	0	0	0	0	1	0
		≥45	1	1	0	0	0	0	0	0	0	0
	Females	<45	12	4	0	0	2	1	1	1	0	3
		≥45	20	2	1	0	3	2	1	0	2	9
Relatives A	Males	<45	62	49	2	4	4	2	1	0	0	0
		≥45	53	40	1	3	4	5	0	0	0	0
	Females	<45	56	46	2	2	3	1	1	0	0	1
		≥45	53	35	0	4	2	5	2	2	1	2
Relatives B	Males	<45	56	47	0	3	3	1	1	0	0	1
		≥45	55	38	2	4	0	7	2	1	0	1
	Females	<45	58	47	4	2	0	1	0	0	1	3
		≥45	44	30	1	4	3	3	0	1	0	2
Controls A	Males	<45	48	39	0	3	2	1	0	0	1	2
		≥45	51	43	1	3	0	2	0	0	0	2
	Females	<45	55	49	0	5	1	0	0	0	0	0
		≥45	29	25	0	2	0	2	0	0	0	0
Controls B	Males	<45	36	34	0	0	1	1	0	0	0	0
		≥45	43	33	0	1	2	1	3	2	1	0
	Females	<45	22	20	0	2	0	0	0	0	0	0
		≥45	37	30	0	0	4	0	2	0	0	1

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM [illegible]

HAEMODYNAMICS IN MYOCARDIAL INFARCTION

RAOUL MALMCRONA

[illegible]

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FROM MEDICAL DEPARTMENT I SAHLGRENSKA SJUKHUSET
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HAEMODYNAMICS IN MYOCARDIAL INFARCTION

by

RAOUL MALMCRONA

GÖTEBORG 1964

The present thesis is based on data presented in the following papers

Haemodynamics in acute myocardial infarction.
Raoul Malmcrona and Ed Varnauskas
Acta Medica Scandinavica, in print 1964.

Haemodynamics during rest and exercise at the end of convalescence from myocardia
infarction. Comparison with earlier and later stages of the disease.
Raoul Malmcrona and Ed Varnauskas.
Acta Medica Scandinavica, in print 1964.

Haemodynamic data during rest and exercise for patients who have or have not been
able to retain their occupation after myocardial infarction.
Raoul Malmcrona, Gun Cramér and Ed Varnauskas
Acta Medica Scandinavica, 174 557 1963

Translated by Klas Magnus Lindskog
Printed by Arboga Boktryckeri AB, Arboga, Sweden, 1964

INTRODUCTION

Myocardial infarction is a prevalent condition which may develop in comparatively young people and take a highly variable course. Its therapeutic management is a major problem. Very comprehensive investigations have been designed to elucidate the pathogenesis of myocardial infarction and some workers have studied its pathophysiology during the acute phase and subsequent course.

At an early stage the circulation was studied experimentally after occlusion of the coronary arteries in open-chest dogs, then in dogs following injection of embolizing agents into the aorta close to the coronary arteries, and most recently after selective embolization of the coronary arteries (25 27 34)

However, because the results of animal experiments are not applicable directly to men successive methods developed for clinical use of measuring blood flow have also been applied to patients with myocardial infarction. Among such methods the following may be mentioned. ballistocardiography (30) Wezler Booger technique for pulse wave analysis (12) the direct Fick method (26) the indicator dilution technique (2, 6, 8, 9 18, 29) and a modification of the latter procedure using a radioactive indicator and external counting (23)

Absolute blood flow values for patients with myocardial infarction vary widely between laboratories. Some discrepancies could be due to different selection of patients, dissimilarities in clinical status between patients examined in the acute phase or differences in time after falling ill or in working capacity or physical activity in patients examined at later stages.

The series of haemodynamically examined patients with myocardial infarction have been small. This is not surprising, for in the acute phase such patients are fragile and, while the patients are kept under observation, every bedside procedure should preferably be done by a team in which each member is responsible for certain tasks which he thoroughly masters. Some of the differences between series could be explained by the small series or in some cases, by reluctance and hesitation in completing all examination procedures with consequent incompleteness and loss of accuracy. Technical facilities have progressively improved, however.

In order to select the treatment of choice for hypotensive states, cardiogenic shock, heart failure and other syndromes often following or complicating myocardial infarction, it is essential to be conversant with as many variations in the haemodynamic pictures exhibited by such patients as possible.

In the present studies the haemodynamic picture of patients with myocardial infarction was examined on one or more occasions during the first three days after onset, in order to establish the natural course of the disease and to investigate how the heamodynamic date were related to the general severity of the condition and to easily measured factors We also wanted to know how the acute course was affected by pyrexia.

To learn more about the subsequent course and restitution after the acute phase of myocardial infarction, the majority of the foregoing patients were also examined before discharge from hospital and before prospective resumption of work, on these occasions both in the reclining and in the sitting posi

abnormal QRS complex remained afebrile All 3 subjects with pathological T waves only had a febrile reaction, two of them sustaining reinfarctions with all ECG mani festations of myocardial infarction. Both subjects not in hospital during the acute phase had a history typical of myocardial infarction. When examined later in hospital one of them had pathological Q waves in leads II, III and aVF while the other had suspected pathological Q wave in lead III, which later seemed normal, ST T changes and signs of coronary failure during exertion. From the time of the attack the latter subject had incessant angina pectoris of great severity

In most patients there was a series of ECG s available for judgement and the development of pathological signs could be followed. Borderline ECG s were directly compared with those of controls (10)

In cases where the patient died and was examined post mortem the diagnosis of myocardial infarction was confirmed.

Those patients who were examined before discharge from hospital and before resumption of work were all men. The average age of the former was 60 years and that of the latter 53 years.

The control series included one group of 12 men averaging 57 years of age who were examined at rest in the reclining position and another group of 14 men averaging 51 years of age who were examined in the sitting position, both at rest and during exertion on a bicycle ergometer (19)

The first control group consisted of ten patients hospitalized for diseases not directly affecting the cardiovascular system and two volunteers. None had chest pain on effort but one complained of exertional dyspnoea. In the second control group all were volunteers None had cardiovascular disease as judged by history physical examination and ECG

Individual data are given in tables in the appendix and in the third paper

Methods

Catheters were inserted percutaneously into the brachial artery and into a cubital vein and advanced centrally The heart rate and arterial pressures were recorded, the cardiac output was determined by dye dilution technic (33) with arterial sampling and — except at examinations in the acute phase — expired air was collected and its oxygen content determined (28) The primary data thus obtained were used for calculation of stroke volume, pulse pressure, arteriovenous oxygen difference peripheral resistance, left ventricular work and left ventricular stroke work.

The methods used are all generally accepted. Some details may however be speci fied Heart rate was determined by measuring the time for 12—15 beats on the ECG A mercury column adjusted to give a pressure corresponding to the systolic arterial pressure was used as reference for arterial pressures The length and width of the ar terial catheter and the transducer used were well suited for measuring arterial pressure The volumes of the arterial samples were equal and the collection time the same for each sample The optical density of each batch of bromsulphalein was measured by spectrophotometry and compared with previ ous samples. The amount of indicator left in the syringe and catheter was measured after each injection. The downslope of the curves was plotted on semilogarithmic paper and extrapolated Hematocrit was measured in arterial blood and no correction was made for trapping of plasma.

Heart rate and arterial pressures were recorded frequently in each patient to show that these were steady Every effort was made to carry out all procedures calmly and quietly and without upsetting the patient.

Cardiac output was measured on two occasions twenty minutes apart in the acute stage. There was no significant difference between these cardiac output determinations between the heart rates recorded at the same time or between the arterial pressures re corded just before. The error of a single determination and the 20 minute variation was estimated from these differences (d) and the number of them (n) by the for mula $\sqrt{\Sigma d^2/2n}$ The error of a single deter mination of heart rate was 3 beats/min, of cardiac output 0.3 l/min, of stroke volume 4 ml, of systolic arterial pressure 5 mm Hg and of diastolic and mean pressure 3 mm Hg, of resistance 1 unit, of left ventricular work 0 5 kpm/min and of left ventricular stroke work 6 pm.

All haemodynamic studies were performed by the author as well as the clinical examina tions in the acute stage and at the follow up studies

All ECG's at rest and during work were reevaluated together with a doctor specially experienced in reading electrocardiograms, and all x-rays of heart and chest were reevaluated together with experienced roent genologists

Four factors were chosen arbitrarily to give an index of the severity of the condition during the acute phase, namely morning temperature of or exceeding 38°C, a serum GOT level exceeding 200 units, ECG evidence of arrhythmia and a systolic blood pressure less than 100 mm Hg at any time during the first three days.

For the investigation designed to elucidate the haemodynamic conditions in working and non working postinfarctive subjects, patients who had been discharged from hospital after treatment for myocardial infarction at least a year previously were summoned to the hospital and subjected to a follow-up examination. In addition they were questioned about their working condi tions before and after the infarction. The information thus obtained was used for evaluation of their working capacity All who had changed from heavier to lighter work and could carry out their new jobs were rated fully capable of working All who received disability pensions on account of their infarction were rated incapacitated. A subject who had been a mechanic before the myocardial infarction was now working as a sweeper at half the wages he had be fore and another who had been a plumber and was working as a filer with much lighter work but had to stay home some days on account of exhaustion were rated incapacitated.

The significance of differences between groups was tested with Student's t and dif ferences on or below the 5 per cent level were regarded significant. Multi variate regressions were analyzed by a university statistician.

Procedure

Patients admitted to the hospital in the morning with distinct signs of myocardial infarction were investigated that afternoon, all others in the morning. Four patients were investigated on all first three days The other patients were investigated on one or two or three consecutive days of the first few days as listed in tables in the appendix. Examinations carried out during the acute phase were done in the ward, all others in the Cardio-pulmonary Laboratory Examina tions before discharge were done with the patients in reclining and sitting positions. Examinations preceding return to work were done with the patients in reclining and sitting positions as well as during gentle exercise on bicycle ergometer. Examina tions of persons with known working capa cities were done with the subjects sitting at ease and operating a bicycle ergometer. The initial determination of cardiac output was

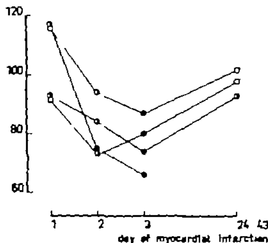


Figure 3 Brachial arterial mean pressure of the same patients as in figures 1 and 2. Same symbols as in these figures.

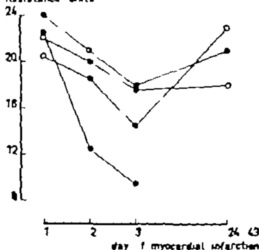


Figure 4 Peripheral resistance of the same patients as in figures 1, 2 and 3. Same symbols as in these figures.

ventricular work and stroke work were lower.

Before resumption of work the circulatory differences between the reclining and sitting positions were similar to those before discharge from hospital except that the oxygen consumptions were the same in the two positions. A tendency to lower arterial pressures persisted.

Circulatory data recorded during light work showed lower cardiac output and stroke volume, higher arteriovenous oxygen difference and lower left ventricular work and stroke work than in controls at the same work load.

Patients retaining work

At rest the only difference in circulatory data between subjects who had returned to work after myocardial infarction and controls was in left ventricular work. These subjects had higher cardiac output and stroke volume, lower arteriovenous oxygen difference and higher left ventricular work and stroke work than patients examined before prospective return to work. They also had higher cardiac output and lower arteriovenous oxygen difference and resistance than patients not retaining work.

During exercise other differences appeared between these subjects and the controls. The subjects' heart rate and arterial systolic pressures increased more and their cardiac output and stroke volume were lower; their arteriovenous oxygen difference was higher and their left ventricular stroke work was lower. Whereas the subjects had lower stroke volume during exercise the controls had higher stroke volume.

Patients not retaining work

The mean age of the group of patients examined when it had become manifest that they would be unable to retain work was higher than the mean age of the controls. By comparison with the controls these patients had lower cardiac output and stroke volume, higher arteriovenous oxygen difference and peripheral resistance and lower left ventricular stroke work. The means of

these factors were of the same order as the corresponding means for the patients examined before prospective return to work.

The groups compared and the differences encountered at the 5 per cent level are depicted in figures 5 6 and 7

Discussion

Studies on dogs

The profound circulatory changes initiated in open-chest dogs by the open chest makes it difficult to analyze the additive effects of the induced myocardial infarction. Similarly much of embolizing material injected into the aorta enters the circulation and embolizes arterioles in the brain, kidneys, skin and bowels, all of which may contribute to the picture of peripheral shock. Hence the reported coronary artery catheterization and selective coronary artery embolization in intact dog was a considerable advance (25 27 34)

Five minutes and thirty minutes after selective embolization of the left coronary artery Hammer and Pitt (16) found reduced stroke volume and cardiac output At the same time there was a decreased mean pressure in the aorta, increased arteriovenous oxygen difference and increased pressure in the left atrium and in the pulmonary artery The total peripheral resistance increased in the majority of dogs. The authors concluded that the initiating event was left ventricular failure with high enddiastolic pressure This led to a build-up of high pressure behind the left ventricle and reduced cardiac output ultimately leading to decrease of the aortic pressure and a compensatory rise of the systematic peripheral resistance

Gunnar et al. (14) analyzed the haemodynamic changes following coronary em-

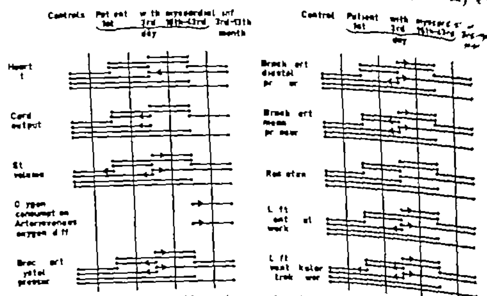


Figure 5 Chart of comparisons of haemodynamic data between controls and groups of patients with myocardial infarction and between the groups of patients. Data recorded at rest recumbent. Groups compared are shown by dots below the group headings and line connecting the dots. Differences at 5 per cent level and higher are indicated by an arrow at the line connecting the dots symbolizing the groups. The arrow points towards the higher mean value. Differences to 3rd day are either to all 3rd day values or to values of patients with temperature less than 38°C on the morning of the 3rd day

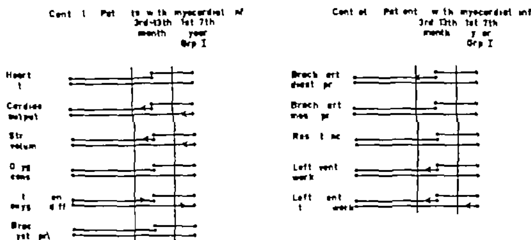
Control Pat ent w th myocard al f
16th–43rd 3rd–13th 1st–7th 3rd 9th
day month year
Grp I Grp II

Heart ot
Ca d ac output
St ok volum
Oxyge ons
A t oven oxyg d ff
Brach art ystol presse

Control Pati t with myoc dial inf
16th–43rd 3rd–13th 1st 7t 3rd–9th
day month year
Grp I Grp II

Brach art d tolic pr sur
Brach art mean pressur
R si tanc
L ft ent lor work
Left ventricul trok work

F gure 6. Chart of comparison of data recorded at rest sitting. "Grp I" patients continuing to work, "Grp II", patients not continuing to work. For explanation of symbols see text to figure 5 D fferences indicated between 16th—43rd day and "3rd—13th month are those in paired observations as well as those between the whole groups.



Figu e 7. Chart f comparisons f data recorded during work on bicycle ergometer load 200 kpm/min. "Grp I patients continuing to work. For explanation f symbols see text to figure 5

bolization in normal, in vagotomized and in atropinized dogs. In the first two groups of dogs they found the same changes as the previous authors, whereas the atropinized dogs exhibited less marked falls in cardiac output and aortic pressure. In another experiment on dogs the same authors (13) demonstrated a spasm in embolized and nonembolized branches of the left coronary artery. Atropine seemed to prevent this spasm. In similar experiments West et al. (34) noticed that the embolized artery showed extinction of the distal part and also that there was reflux of contrast medium to non-embolized vessels. However, the initial pattern appeared unchanged after atropenization or the use of vasodilator drugs. The initial effect was followed by vasodilatation and a gradual return towards the normal pattern. Measurements of the coronary sinus blood flow showed a decrease at the first minute followed by an increase to values well over control values for about 8 minutes.

Rushmer et al. (27) stressed that there was a reduction in peak ejection velocity and peak acceleration of blood in the aorta and a reduced stroke volume after occlusion of coronary arteries in dogs.

Experiments on dogs have thus demonstrated signs of left ventricular failure after coronary occlusion. Peripheral resistance rose only when the arterial pressure dropped. The experiments in question were not extended over any considerably length of time, and whether occlusion of a coronary artery is accompanied by instantaneous development of a reflex spasm in the peripheral branches of the same coronary vessel or in other coronary vessels is not yet known.

Studies on patients

Measurements on human subjects (2, 6, 8, 9 18, 23 26, 29) have shown that in the acute phase of myocardial infarction the stroke volume and cardiac output is more or less reduced, the extent of the reduction being well correlated with the patient's clinical status. The absolute blood flow values show however wide variations between laboratories for patients with myocardial infarction as well as for normals (32). Though the peripheral resistance has been increased in the majority of patients, it has been lower in some.

Intracardiac pressure have not been measured in the acute phase of myocardial infarction, but in the subacute phase Müller and Rørvik (24) measured the pressures in the right heart. They reported that patients with signs of heart failure had increased pulmonary wedge pressure and somewhat reduced cardiac output and that patients with no failure but angina pectoris had normal pulmonary wedge pressure, rising to excessive levels during exercise, and normal cardiac output.

Finding a raised central venous pressure in myocardial infarction, Lee (18) suggested that dilatation of the left ventricle might impair right ventricular function.

Patients and controls of this study

Haemodynamic studies have to be performed in small selected groups of patients. Small groups of patients may differ in several respects even if rather rigid criteria are used for their selection. This may account for part of the differences between the patients of this study and those of other investigations. The smallest groups of patients of this study those of patients with temperature less than 38°C on 1st and 3rd day of infarction or higher temperature on 3rd day were, however, very similar with respect to age, height and weight. The mean temperature of the group with low temperature on 1st day of infarction was, however 36.9°C while it was 37.5 in the group regarded as having low temperature on 3rd

day The mean of the highest serum GOT found at routine examinations 2—4 times at appropriate time intervals after patients admission to hospital was 170 units in the 1st day group and 347 and 306 units respectively in the 3rd day groups. These differences may have influenced the haemodynamic data recorded in patients with low temperature on 1st and 3rd day On 1st day higher cardiac output may be found as those patients may not have as large infarctions as the others. On 3rd day the peripheral resistance may be influenced by the small temperature rise making it possible for the heart to forward a higher output.

The age difference between the groups investigated during the first hospital stay and the groups investigated before intended return to work was inherent in the clinical material available. The older subjects of the first mentioned group had already retired and could not be included in the second group. On account of the age difference comparison was also made between the values of same patients recorded before they left hospital and before they intended to return to work.

When comparing the patients investigated in the earlier stages of myocardial infarction with those investigated when their working capacity after the infarction could be assessed it may be kept in mind that they are a sample of patients treated some years earlier in the hospital. The diagnosis could not be based on exactly the same criteria, fewer ECG's were available, determinations of serum GOT were not made and I have only seen some of them in the acute stage myself However most of them were seen by me at an earlier reexamination (20)

The reason for the age difference between the patients retaining and not retaining work is that most of them are from the same original sample and younger people tend to retain their work while older ones do not.

The two groups of controls were collected to fit as well as possible to the different groups of patients with myocardial infarction. When collecting the younger group of controls certain care was taken only to include persons in full health and persons with and without physical work. The higher aged group of controls was included for comparison with the acute stage patients. In patients with diseases not directly affecting the cardiovascular system seemed most appropriate

The highest age difference between groups compared was 7 years and encountered between controls or patients retaining work and patients not retaining work.

In the age category under discussion the cardiac output reduction per decade is approximately 0.5 l/min according to Brandfonbrener et al. (1) From 25 to 50 years of age, however they found a 1.5 l/min decrease in cardiac output, while the difference between groups of normals with mean ages of 26 and 51 years at this laboratory was 1.0 l/min only The assumption that the cardiac output differences demonstrated between the controls and the two above mentioned groups of patients were essentially due to factors other than age is borne out by the fact that the groups also exhibited unequal arteriovenous oxygen differences.

Methods used in this study

It is often suggested that investigations of this kind should not be performed in severely ill patients. The patients may however be disturbed less than by checking the blood pressure by sphygmomanometer The methods imply a very good supervision of the patient and the data collected may in every patient lead to better founded clinical measures

The hazards of intraarterial catheters may be directly contrasted to what may be gained

by their use. The patients investigated in the acute stage were severely ill and if the disease would take an unfavourable course every one would benefit from accurate measurements of the blood pressure and appropriate measures based on them. After the catheters have been inserted blood pressure readings can be made almost continuously without disturbing the patients.

For evaluation of blood pressure response to work intraarterial readings are necessary The intraarterial catheter will also provide a wider choice of indicator dilution methods for cardiac output determinations.

It was felt that an indicator dilution technic using direct arterial sampling was the most accurate method available.

As we wanted to make many cardiac output determinations after each other in some of the patients we chose bromsulphalein as indicator It is suitable for use in an indicator dilution technique in that it mixes completely with the blood and remains in the circulation long enough to record the dilution curve. It is easily measured in plasma samples by spectrophotometry and the curve may be calibrated from these concentrations. The loss from the circulation is such that consecutive cardiac output determinations may be made at short intervals.

Acute stage

In the present investigation the stroke volume was low as early as the first day of myocardial infarction and the cardiac output was low on the third day unless fever altered the haemodynamics. These findings bear out previously reported findings in experiments on dogs and investigations on human subjects.

The diminution in stroke volume and cardiac output may be the result of a myocardial failure or it may be a consequence of bed rest. No certain information is available on the effect of bed rest on cardiac output but it may be mentioned that 7 of the 12 controls examined in the reclining position had been staying in bed for one day or more prior to the cardiac output determination but their cardiac output did not differ from that of the other 5 controls All who have investigated patients with myocardial infarction have also found an increase of cardiac output towards the end of hospital stay when the patients are still at rest even if not in bed. Most investigators have also found a relation between the severity of the disease as judged by clinical features and the decrease of cardiac output and stroke volume.

A failure of the heart as a pump after myocardial infarction may simply be the consequence of a mechanical defect in the myocardial function due to inactivity of the infarcted muscle. There may at the same time be inflammatory reactions in the surrounding tissue interfering with its function. The mechanic effects may extend from slight interference with the normal harmonious combination of forces during systole to inverse movements of large parts of the cardiac walls or septum. Widespread coronary atheromatous disease may at the same time be responsible for diminution in blood supply to the whole heart and the effect may be exaggerated during conditions of systemic hypotension. There may also be spasm in not occluded arteries (13) or there may be vasodilatation (34)

One way to test the myocardial ability would be to test if the infarcted heart can respond by an increased output to a stimulus normally inducing an increase. No direct tests with this aim have been performed in the acute stage of myocardial infarction either in previous studies or in this study Lee (18) however found a heart failure blood pressure response to the Valsalva manoeuvres in patients after acute myocardial infarction Another way would be t[illegible]

analyze the arterial pressure curves as Rushmer (27) did in experiments on dogs. No such analysis has, however, yet been undertaken on patients

If a moderate decrease of cardiac output is a disadvantage for the heart itself or other organs in the body is not known. Analysis of excess lactate, pH and arteriovenous oxygen difference at the same time as cardiac output is determined may help to solve this problem in patients with myocardial infarction.

When fever supervenes the picture changes markedly because the peripheral resistance then diminishes and gives rise to compensatory mechanisms tending to increase the cardiac output. The net result is that the latter remains unchanged and is of the same order as in controls.

Fever may have been responsible for the low peripheral resistance previously reported in some patients with myocardial infarction and may explain why postinfarctive shock has also been ascribed to peripheral vasodilatation (5)

In healthy young males pyrogen-induced fever is accompanied by reduced peripheral resistance and arterial pressure and increased cardiac output (11) Afebrile patients with myocardial infarction have unchanged peripheral resistance, reduced cardiac output and reduced arterial pressure. Patients with myocardial infarction and fever have reduced peripheral resistance, unchanged cardiac output and reduced arterial pressure. The common denominator for these patients seems to be myocardial failure leading to inability to maintain the arterial pressure When the peripheral resistance is unchanged, cardiac output falls when the peripheral resistance is lower cardiac output may be maintained.

Previous investigators have found that the peripheral resistance is moderately or markedly enhanced in most patients after infarction. This may be due to dissimilar groups of patients The patients of other investigators may have been more severely ill or they may have been investigated when afebrile. The temperature has not been reported by any previous investigator

Finally it may be emphasized that the changes observed following a temperature rise in patients with myocardial infarction may as yet only be regarded as suggestive as the groups compared are small The results, however clearly demonstrate the possibility of profound differences in the haemodynamics of patients with different patterns of the response of the body to the infarction They thereby demonstrate the need of further studies. It seems essential to perform serial measurements on afebrile patients with myocardial infarction whose clinical course pass from a satisfactory general condition to a state of cardiogenic shock.

The change of the circulation induced by moderate fever seems not to put a further strain on the myocardium even if the output is higher than in patients without fever A great part of the vasodilatation may however have taken place in skinvessels and the distribution of blood may be unfavourable compared with the distribution in patients without fever Efforts to decrease a very high body temperature, which in itself disturbs the patient, by salicylates may lead to an even more unfavourable distribution of blood during sweathing. To decrease body temperature by cold packings and cold air streams is more likely to be the way to do it if necessary Another approach would be to try to decrease the body's reaction to the infarction by corticosteroids.

Efforts to decrease a very high peripheral resistance in patients without fever in order to improve their circulation may also be hazardous as an increase in cardiac output and unchanged arterial pressure and perfusion pressure of the coronary arteries can not be guaranteed. Anyhow to place a

patient with an unfavourable high resistance, if it is possible to ascertain what is an unfavourable high resistance, in a hot and dry environment would be the best way to do it. When Carlsten et al. (4) moved 6 normal young males from 20°C to 37°C dry environment they found a decrease in resistance in all of them. The cardiac output rose in five. There were a lower mean brachial arterial pressure and a lower mean pulmonary arterial pressure in four. In all the arteriovenous oxygen difference was smaller in the warmer surrounding.

Clinical considerations

Heart rate can almost always be counted and systolic and diastolic arterial blood pressure are often easily determined at the bedside by a sphygmomanometer. They are, however, only poorly correlated to more fundamental physiologic phenomena, cardiac output, stroke volume and total peripheral resistance. Hence it would be highly advantageous if the latter instead could be measured directly by routine bedside procedures. The limiting factors are the need of expensive apparatus, experienced people to use them and a larger technical staff than is now available in most hospitals. There is also the arteriopuncture with its attendant risk of complications and uncertainty with respect to how long catheter safely may be allowed to dwell in an artery. This drawback can be circumvented by the use of the isotope dilution technique with external counting (23) or of ear oximetry (7) and calibration at the tail of the dye dilution curve. The arterial pressure can, however, be measured much more accurately through an indwelling arterial catheter. Changes in blood flow may be screened by skin temperature measurements. The blood flow through skin and muscles can be determined independently at the bedside with the aid of plethysmography.

When a patient with myocardial infarction becomes critically ill one must often distinguish between the following; thromboembolic complications, arrhythmias, heart failure or heart failure complicated by change of the peripheral circulation. Thromboembolic complications are nowadays to a large extent avoidable by early mobilization and anticoagulant therapy. Arrythmias could be detected and treated sooner if cardioscopes with automatic monitoring and alarm devices were available for a greater number of patients (3, 15). The possible development of heart failure and changes in the regulation of peripheral resistance could be followed using technics mentioned above and treatment could, if necessary, be instituted on better knowledge of the haemodynamic state of the individual patient. Treatment would then be much better founded than is now often the case.

There is need for improved diagnosis and treatment in cases of myocardial infarction with complications or hazardous course. Pilot studies on these lines have been made in which such patients were thoroughly followed during treatment with metaraminol or digitalis (21, 22).

Subacute stage

The stroke volume and cardiac output was higher at the end of hospital stay than in afebrile patients on third day. This indicates that the heart itself comparatively soon recovers at least partly from the trauma of the infarction. These is borne out by the essentially normal circulatory response of these patients to postural changes.

That the circulation remains affected despite the rapid recovery from the acute trauma is evident from the results obtained when patients were examined prior to return to work after myocardial infarction. The

analyze the arterial pressure curves as Rushmer (27) did in experiments on dogs. No such analysis has, however, yet been undertaken on patients.

If a moderate decrease of cardiac output is a disadvantage for the heart itself or other organs in the body is not known. Analysis of excess lactate, pH and arteriovenous oxygen difference at the same time as cardiac output is determined may help to solve this problem in patients with myocardial infarction.

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Finally it may be emphasized that the changes observed following a temperature rise in patients with myocardial infarction may as yet only be regarded as suggestive as the groups compared are small. The results, however, clearly demonstrate the possibility of profound differences in the haemodynamics of patients with different patterns of the response of the body to the infarction. They thereby demonstrate the need of further studies. It seems essential to perform serial measurements on afebrile patients with myocardial infarction whose clinical course pass from a satisfactory general condition to a state of cardiogenic shock.

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APPENDIX

Tables of clinical data and haemodynamic values of individual patients and control subjects

Table 1 *Clinical data of patient in acute*

Patient	Sex	Age, years	Height, cm	Weight, kg	Previous disease related to myocardial infarction	Days with morning temps. over 37°C and 38°C resp.	Serum GOT	Heart rhythm	Electrocardiographic signs of myocardial infarction
1	2	3	4	5	6	7	8	9	10
1	F	69	161	57	Hypertension since 10 years	11-0	85	Sinusrhythm	ST-segment elevation and pathological Q wave in II III VF and CR_4 and CR_5
2	M	66	166	53	None	3-0	154	Sinusrhythm	ST-segment elevation and pathological T wave in CR_4 and IVR
3	M	45	179	90	None	6-2	316	Sinusrhythm	ST-segment elevation and pathological Q wave in aVF II, III and CR_5
4	M	65	186([1])	93([1])	None	8-4	206	Sinusrhythm with ventricular premature beats	ST-segment elevation and pathological Q wave in CR_4 and CR_4
5	M	74	174([1])	68([1])	Hypertension since 30 years	3-0	670	Sinusrhythm, bundle branch block, total atrioventricular heart block	ST-segment elevation and pathological Q wave in CR_5 and CR

([1]) Registered at topsy

and subacute myocardial infarction.

Blood pressure levels during hospital stay	Symptoms or signs of heart failure during hospital stay	Heart size in subacute stage, total volume and volume per sqm BSA, ml	Roentgenological signs of pulmonary congestion in subacute stage	Days f investigation	Morning temp. t day of investigation, °C	Digitalis given before haemodynamic study	Status during haemodynamic study	Remarks
11	12	13	14	15	16	17	18	19
160/80 110/70 140/90	No	620/380	No	3rd 4th 5th 23rd	38.0 37.6 37.6 36.9	No N No No	Good Good Good Good	Full working capacity soon after hospital stay Followed 2 years and 2 months
120/80	No	760/480	N	7th 8th 27th	36.6 36.6 36.4	No No No	Good Good Good	Uneventful recovery Followed 5 years
120/80	Yes	1090/570	Yes	4th	38.4	Yes	Good	Full working capacity 1 years later Followed 2½ years
120/80	No	—	—	3rd	38.2	Yes	Good	Died on 19th day
120/80 80/60 last 12 hours	Yes	—	—	2nd	37.7	No	Good	Died on 3rd day

Table 1 continued *Clinical data of patient in acute*

1	2	3	4	5	6	7	8	9	10
6	M	67	169(1)	72(1)	None	10-4	430	Sinusrhythm	ST-segment elevation and pathological Q wave in CR
7	M	59	167	75	None	1-0	460	Sinusrhythm	ST-segment elevation and pathological Q wave in CR—CR
8	M	58	179(1)	68(1)	None	3-2		Sinusrhythm, bundle branch block, total atrioventricular heart block	ST-segment elevation and pathological Q wave in CR and CR
9	M	58	172	67	None	10-2	185	Sinusrhythm	ST-segment elevation and pathological Q wave in aVF II III and CR
10	M	56	176	79	None	5-0	240	Sinusrhythm	ST-segment elevation and pathological Q wave in aVF II and III
11	M	75	170	77	Effort angina	6-0	41	Sinusrhythm	ST-segment elevation and pathological Q wave in CR and CR
12	M	50	163	69	Diabetes mellitus since 10 years, myocardial infarction 5 month before actual one	16-4	225	Sinusrhythm	ST-segment elevation and pathological Q wave in CR—CR

(1) Registered autopsy

and subacute myocardial infarction.

11	12	13	14	15	16	17	18	19
180/110 95/ 120/80	Yes	—	—	3rd	38.7	No	Pains, cold sweat	Died on 1[illegible] day
160/90 120/80	N	760/410	No	3rd	37.5	No	Good	Returned to work after [illegible] months. Died at [illegible] 6 months later
190/120 100/70	Yes	—	—	2nd	37.7	No	Pains	Died on 4[illegible] day
150/80 100/70 160/80	Yes	570/320	N	1st 2nd	37.1 39.6	No No	Pains Pains and cold sweat	Returned to work after 6 months Followed 1 year
				3rd 43rd	38.1 —	Yes No	Good Good	
160/100 110/70 120/80	No	990/490	N	31st	36.5	No	Good	Effort angina, Returned to work after 6 months Followed 1½ years
160/100 150/80 190/110	Yes	1260/670	Yes	29th	36.8	N	Good	Readmitted to hospital after 3 months in pulmonary oedema Recovered
170/100 120/80 170/100	N	630/350	No	28th	37.0	No	Good	Effort angina Returned to work after 8 months Followed 3 years

Table 1 continued *Clinical data of patients in acute*

1	2	3	4	5	6	7	8	9	10
21	M	52	167	67	Hyperlipemia	0-0	75	Sinusrhythm	ST-segment elevation and pathological Q wave in CR
22	M	69	—	—	None	10-5	199	Sinusrhythm	ST-segment elevation and pathological Q wave in I and CR—CR
23	M	47	176	75	None	10-1	412	Atrial fibrillation, sinusrhythm	ST segment elevation and pathological Q wa e in aVL, CR and CR
24	M	49	190	76	None	0-0	132	Sinusrhythm	ST-segment elevation and pathological Q wave in CR and CR
25	M	51	180	81	Effort angina since 6 months	20-4	238	Sinusrhythm	ST-segment elevation and pathological Q wave in aVF II III, CR and CR
26	M	63	172	66	Diabetes mellitus since 6 years	5-0	124	Sinusrhythm	ST-segment elevation and pathological Q wave in VF I and II
27	M	61	166	74	Hypertension since 3 years	18-0	320	Sinusrhythm	ST-segment elevation and pathological Q wave in CR and CR
28	M	77	164	71	None	5-2	175	Sinusrhythm, ventricular premature beats	ST-segment levation and pathological Q wa e in aVF II and III

and subacute myocardial infarction.

11	12	13	14	15	16	17	18	19
130/80 160/90	No	710/410	No	2nd 3rd 23rd	36.5 36.5 36.5	No No N	Good Good Good	Returned to work after 5 months Died of carcinoma of the lung 6 months later
55/ 130/80 90/70 110/70	Yes	—	—	2nd	38.1	No	Good	Progressive heart failure Died on 17th day
120/100 90/70 110/70	No	790/320	N	2nd 3rd	38.0 37.8	Yes Yes	Good Good	Uneventful recovery Returned to work after 5 months Followed 2 years
140/80 110/70 130/80	N	610/300	No	1st 2nd 19th	36.7 36.8 —	No N No	Good Good Good	Returned to work after 6 months Followed 1½ year
150/90 110/80 140/100	Yes	820/410 920/460	Yes	2nd 3rd	37.0 38.8	No No	Good Good	Returned to work after 8 months Effort angina and heart failure. Died in reinfarction 1½ year later
140/80 120/80 140/80	N	730/410	No	2nd 3rd 24th	37.4 37.8 36.6	No No No	Good Good Good	Returned to work after 5 months Followed 1½ year
160/100 100/70 120/80	No	770/440	No	2nd	37.6	No	Pallor, cold- sweat	Returned to work after 7 months Followed 1½ year
140/90 100/70 120/80	No	1030/600	No	1st 2nd 3rd 25th	36.6 37.8 38.2 36.8	No No No No	Good Good Good Good	Uneventful recovery Died in reinfarction after 8 months

Table 1 continued

Clinical data *f patients in acute*

1	2	3	4	5	6	7	8	9	10
29	M	56	170	75	None	6-1	444	1st degree atrioventricular heart block, sinusrhythm	ST-segment elevation and pathological Q wave in aVF II and III
30	M	59	184	77	None	4-1	450	Sinusrhythm, supraventricular premature beats, atrial fibrillation	ST-segment elevation and pathological Q wave in CR₃ and CR₄
31	M	46	175	77	None	3-1	440	Sinusrhythm	ST-segment elevation and pathological Q wave in VL, I, CR₃ and CR₄
32	M	59	175	76	Hypertension since 2 years	3-1	176	Sinusrhythm	ST-segment elevation and pathological Q wave in I and CR₂—CR₅
33	M	57	175	80	None	2-0	440	Sinusrhythm	ST-segment elevation and pathological Q wave in aVF II and III
4	M	49	186	81	None	7-4	220	Sinusrhythm, total atrioventricular heart block, sinusrhythm	ST-segment elevation and pathological Q wave in aVF and III
	M	39	170	70	None	2-0	142	Sinusrhythm	ST-segment levation and Pathological Q wa in aVF and III

and subacute myocardial infarction.

11	12	13	14	15	16	17	18	19
70/ 150/100 120/70	No	950/310	Yes	1st 2nd 3rd 24th	36.6 37.4 38.6 36.7	No No N No	Good Good Tired Good	Heart failure Returned to work after 5 months Died in reinfarction 3 months later
130/80 100/70 110/70	No	820/420	No	2nd 3rd 4th 32nd	37.4 38.0 38.2 36.6	No No N Yes	Good Good Good Good	Returned to work after 6 months Followed 1 year
160/100 110/80 120/80	Yes	840/440	Yes	3rd 4th 35th	38.1 38.0 36.5	No No No	Good Good Good	Returned to work after 5 months Followed 1 year
160/100 100/80	No	860/450	N	4th 25th	37.8 37.3	No No	Good Good	Returned to work after 5 months Followed 5 months
130/100 120/80 150/100	No	830/440	No	2nd 3rd 17th	37.4 37.6 36.5	No No No	Good Good Good	Returned to work after 4 months Followed 1 year
70/40 100/70 130/90	No	830/410	No	1st 27th	36.8 36.6	No No	Palms Good	Effort angina Returned to work after 3 months Followed 1½ year
120/80 90/60 130/80	No	640/350	No	2nd 23rd	37.2 36.5	No No	Good Good	Returned to work after 6 months Followed 1 year

Table 1 continued *Clinical data of patients in acute*

1	2	3	4	5	6	7	8	9	10
36	F	73	134	70	Hypertension known 6 months Mitral insufficiency	13 2	174	Atrial fibrillation	ST-segment elevation and patological Q and T waves in aVF II and III
37	M	49	—	—	None	5-1	220	Sinusrhythm	ST-segment levation and patological Q and T waves in VL, I II and CR₂—CR₅
38	M	72	163	67	None	4-0	73	Sinusrhythm	Patological Q and T waves in aVF and III
39	M	59	184	64	None	8-4	180	Sinusrhythm	ST-segment elevation and patological Q wave in aVF II III and CR₂—CR
40	M	70	163	56	None	6-1	567	Sinusrhythm	ST-segment elevation and patological Q wave in CR₂ and CR₄
41	M	63	170	73	None	14-2	300	Sinusrhythm, atrial fibrillation 2 hours, sinusrhythm	ST-segment elevation and patological Q wave in CR₂—CR₅

and subacute myocardial infarction.

11	12	13	14	15	16	17	18	19
160/50 110/60 150/80	No	1070/640	Yes	2nd 29th	38.6 36.4	N No	Good Good	Left hospital after 1 month Readmitted 4 months later with signs of cerebral embolism
120/90	No	—	—	1st 2nd	37.1 38.7	No No	Good Pains, cold-sweat	Died on 10th day
200/110 70/ 90/60 160/90	No	750/440	No	1st 2nd	36.0 37.3	N No	Good Good	Uneventful recovery Followed 1 year
105/65 100/70 115/70	No	750/400	No	3rd 23rd	39.0 37.0	No No	Good Good	Returned to work after 5 months Followed 1 year
150/110 90/70 120/70	No	880/550	Yes	3rd 25th	37.4 36.8	No Yes	Pains, cold-sweat Good	Uneventful recovery Followed 4 months
140/95 90/ 120/70	No	1010/550	Yes	3rd	38.2	No	Good	Effort angina and heart failure Died in reinfarction after 8 months

Table 2 *Haemodynamic data of patients in acute myocardial infarction*

Patient	Day of myocardial infarction	Heart rate beats/min	Cardiac output l/min	Stroke volume ml	Brachial arterial pressure			Resistance units	Left ventricular work kpm/min	Left ventricular stroke work gm
					systolic mm Hg	diastolic mm Hg	mean mm Hg			
1	2	3	4	5	6	7	8	9	10	11
1(¹)	3rd	82	3.7	45	113	63	92	25	4.6	56
	3rd	84	3.5	41	124	65	86	25	4.1	48
	4th	79	4.3	54	107	51	75	18	4.4	55
	4th	80	4.4	55	118	52	79	18	4.7	59
	5th	78	4.0	52	118	55	78	19	4.3	55
	5th	76	3.8	50	111	52	75	20	3.9	51
2	7th	66	4.3	66	115	62	86	20	5.1	77
	8th	71	3.9	55	117	68	92	24	4.9	69
	9th	70	4.9	70	123	63	85	17	5.7	81
3	4th	73	6.1	83	105	69	83	14	6.9	94
	4th	75	5.3	71	106	70	84	16	6.1	81
4	3rd	112	7.3	65	116	62	81	11	8.1	72
	3rd	112	7.1	64	109	60	78	11	7.6	68
5	2nd	82	4.3	52	110	73	87	21	5.0	62
	2nd	82	4.2	51	104	67	80	19	4.6	56
6	3rd	78	6.2	79	117	68	93	15	7.5	97
	3rd	85	6.2	73	112	62	81	13	6.8	80
7	3rd	65	4.5	68	121	70	90	20	5.5	83
	3rd	71	4.2	58	132	73	102	25	5.8	81
8	2nd	94	6.0	64	141	92	111	19	9.1	97
	2nd	95	4.5	47	130	84	105	24	6.4	67
9	1st	72	5.0	70	165	87	119	24	8.2	113
	1st	73	5.5	75	161	83	114	21	8.5	116
	2nd	91	6.1	67	105	56	75	12	6.2	68
	2nd	96	6.0	63	109	57	73	13	6.1	64
	3rd	94	7.4	78	96	49	67	9	6.7	71
	3rd	88	6.6	75	92	50	65	10	5.8	66

Table 2 continued Haemodynamic data of patients in acute myocardial infarction.

1	2	3	4	5	6	7	8	9	10	11
14	1st	76	5.8	77	117	70	90	15	7.2	94
	1st	76	6.1	81	115	72	90	15	7.5	99
	2nd	91	7.6	83	120	67	84	11	8.7	95
	2nd	91	7.6	83	111	56	81	11	8.3	91
15	2nd	83	4.2	51	127	71	99	23	5.7	69
	2nd	79	4.2	53	132	73	100	24	5.7	72
	3rd	76	4.3	56	116	64	81	19	4.7	62
	3rd	79	3.8	49	117	69	92	24	4.8	61
16	1st	62	4 9	79	122	63	85	17	5.7	92
	1st	71	5.2	73	124	64	90	17	6.3	89
17	1st	48	4.8	100	94	52	70	13	4.6	95
	1st	47	5.1	108	96	52	70	14	4.8	103
	2nd	52	4.7	91	98	50	69	15	4.5	86
	2nd	54	5.5	102	99	54	71	13	5.3	97
	2nd	57	5 4	95	105	57	73	14	5.4	94
18	1st	97	5.6	58	129	77	96	17	7.3	76
	1st	102	5.4	53	120	75	95	18	7.0	69
	2nd	97	4.8	50	96	62	75	16	4.9	51
19	2nd	83	4.0	48	94	46	70	18	3.8	46
	2nd	85	4.6	54	103	52	72	16	4.8	53
	3rd	76	5.3	69	106	49	71	14	5 1	67
	3rd	82	5.5	67	117	50	75	14	5.6	68
20 [1]	1st	60	3.9	64	128	65	93	24	4.9	81
	2nd	59	4 1	69	121	66	84	21	4 7	79
	3rd	73	4.0	55	93	59	74	18	4 1	55
21	2nd	69	5.5	80	123	58	87	16	6 5	95
	2nd	66	5 4	82	132	62	90	17	6.6	100
	3rd	56	4.2	74	125	63	93	22	5.3	94
	3rd	57	4.1	72	127	64	84	21	4.7	82
22	2nd [2]	103	4.8	47	112	65	67	18	5.7	56
	2nd	99	4.7	47	111	65	83	18	5.3	53
23	2nd	91	5.1	56	109	68	85	17	5.9	65
	2nd	95	5.4	57	101	63	78	14	5.7	61
	3rd	85	4 9	57	108	61	78	16	5.2	61

[1] Female.
[2] This patient was investigated 30 hours after onset illness and in error in paper I treated as investigated within 24 hours.

Table 2 continued Haemodynamic data of patients in acute myocardial infarction

1	2	3	4	5	6	7	8	9	10	11
24	1st(*)	59	11.0	186	136	67	98	9	14.7	248
	1st(*)	58	10.6	183	140	70	100	9	14.4	249
	2nd	57	7.4	130	115	58	78	11	7.9	138
	2nd	57	7.4	130	111	56	77	10	7.7	136
25	2nd	72	4.7	65	115	70	89	19	5.6	79
	2nd	86	4.8	56	128	78	94	20	6.2	72
	3rd	108	5.4	50	119	72	90	17	6.6	61
	3rd	109	5.0	46	119	74	91	18	6.2	57
26	2nd	86	5.4	63	124	57	88	16	6.5	75
	3rd	84	6.3	76	100	45	80	13	6.9	83
	3rd	84	6.9	82	122	59	84	12	7.8	94
27	2nd	102	5.5	54	130	73	92	17	6.9	68
	2nd	105	6.7	63	128	72	92	14	8.3	79
28	1st	49	4.5	91	135	63	93	21	5.7	115
	1st	53	4.6	87	140	65	93	20	5.8	110
	2nd	44	3.8	86	115	49	74	20	3.8	87
	2nd	47	4.3	90	113	48	72	17	4.2	88
	3rd	59	5.8	99	127	53	80	14	6.4	108
	3rd	57	5.4	95	119	52	80	15	5.9	103
29	1st	76	5.2	69	151	90	119	23	8.4	112
	1st	83	5.2	62	148	78	112	22	7.9	94
	2nd	61	4.8	79	133	66	91	19	5.9	98
	2nd	64	4.6	72	139	62	96	21	6.0	94
	3rd	78	5.0	65	115	57	84	17	5.8	74
	3rd	75	4.7	63	137	68	90	19	5.8	77
30	2nd	78	5.4	70	100	55	72	13	5.3	69
	2nd	75	5.7	77	96	55	74	13	5.8	78
	3rd	84	5.5	66	107	63	80	15	6.0	72
	3rd	78	5.7	73	111	68	83	15	6.4	82
	4th	145(*)	3.5	24	78	50	58	17	2.7	19
	4th	146(*)	3.4	23	75	52	60	18	2.7	19
31	3rd	103	5.5	54	111	57	74	13	5.6	54
	3rd	107	5.7	53	103	58	77	14	5.9	56
	4th	97	5.1	52	100	55	73	14	5.0	52
	4th	94	5.2	56	103	60	74	14	5.3	56
32	4th	94	5.8	62	123	74	96	16	7.6	81
	4th	93	5.8	62	120	69	90	16	7.1	76

(*) Omitted from mean value calculations in p per I
( ) Atrial fibrillation.

Table 2 continued Haemodynamic data of patients in acute myocardial infarction

1	2	3	4	5	6	7	8	9	10	11
33	2nd	81	5.3	66	126	66	90	17	6 5	81
	2nd	83	5.5	66	123	65	90	17	6.7	81
	3rd	84	5.0	60	100	53	72	14	4 9	59
	3rd	82	5.4	66	108	56	80	15	5 9	77
34	1st	47(*)	4 0	85	112	50	65	16	3.5	75
	1st	46(*)	4.3	92	105	45	62	15	3.6	78
35	2nd	48	4.3	89	95	44	63	15	3.7	76
	2nd	47	4.0	86	104	50	70	17	3 9	82
36(*)	2nd	53(*)	2.5	48	112	37	60	24	2.1	39
	2nd	53(*)	2 6	48	132	41	60	23	2.1	39
37	1st	111	6.7	60	120	80	99	15	9.0	81
	1st	112	6.7	60	124	74	98	15	9.0	80
	2nd	110	7.0	64	111	67	88	13	8.4	77
	2nd	112	6.3	57	107	65	85	13	7.3	66
38	1st	79	4.6	59	103	36	63	14	4.0	51
	1st	78	5.5	70	109	38	58	11	4.3	55
	2nd	84	5.0	59	132	60	80	16	5.4	64
	2nd	79	5.3	67	140	46	90	17	6.5	82
39	3rd	131(*)	4 4	34	97	51	64	14	3 9	30
	3rd	119(*)	4 4	37	93	51	63	14	3 8	32
40	3rd	104	3 1	30	87	37	60	19	2 5	25
	3rd	103	3 4	33	85	40	58	17	2.7	26
41	3rd	78	5.5	70	95	54	71	13	5.3	68
	3rd	79	6.5	82	97	53	74	11	6.5	83

( ) Female
(°) Atrial fibrillation.
(*) Total atrioventricular heart block.
(*) Sinusrhythm with supraventricular premature beats.

Table 3 *Haemodynamic data of patients in*

Patient	Days after myocardial infarction	Body position	Heart rate beats/min	Cardiac output l/min	Stroke volume ml	Oxygen consumption ml/min
1	2	3	4	5	6	7
1(¹)	24	Recumbent	79	5.1	65	179
		Sitting	77	4.3	55	179
		Sitting	73	4.1	56	176
2	27	Recumbent	65	4.7	72	—
9	43	Recumbent(²)	81	11.2	139	271
		Sitting	81	7.6	94	283
		Sitting	77	7.2	94	278
10	31	Recumbent	88	7.7	87	289
		Recumbent	77	7.4	96	289
11	29	Recumbent	70	6.0	85	260
		Recumbent	71	6.1	85	252
12	28	Recumbent	103	5.6	54	—
13	28	Sitting	95	6.0	63	293
		Sitting	81	5.2	65	267
15	28	Recumbent	71	4.4	61	189
		Sitting	77	4.3	56	191
		Sitting	75	4.0	54	201
20(¹)	24	Recumbent	83	4.3	52	154
		Sitting	75	3.6	49	147
21	22	Recumbent	61	5.9	97	194
		Sitting	56	4.5	80	209
		Sitting	54	4.2	79	180
24	18	Sitting	57	5.9	104	303
		Sitting	59	6.0	102	268

(¹) Female.
(²) Omitted from mean value calculations in paper I

…bacute myocardial infarction

Arteriovenous oxygen difference ml/l	Brachial arterial pressure: systolic mm Hg	Brachial arterial pressure: diastolic mm Hg	Brachial arterial pressure: mean mm Hg	Resistance units	Left ventricular work kgm/min	Left ventricular stroke work gm
8	9	10	11	12	13	14
35	198	89	126	25	8.8	111
42	176	85	116	27	6.7	87
43	184	87	115	28	6.4	88
—	164	95	120	25	7.7	118
24	150	69	96	9	14.7	182
37	165	76	110	14	11.4	141
39	145	70	102	14	10.0	130
38	145	83	104	14	10.8	123
39	134	80	100	14	10.1	131
43	170	78	111	19	9.0	128
42	173	82	117	19	9.7	135
—	157	88	119	21	9.0	87
49	125	65	86	14	7.0	74
51	108	59	75	14	5.3	66
43	133	73	97	22	5.7	81
45	134	73	97	23	5.7	74
50	131	70	95	24	5.2	70
35	116	73	93	21	5.5	66
40	108	67	87	24	4.3	53
33	145	79	101	17	8.1	[illegible]
47	128	66	87	19	5.3	[illegible]
42	130	67	86	20	5.0	[illegible]
31	120	66	88	15	7.?	[illegible]
44	119	67	90	15	[illegible]	[illegible]

Table 3 continued *Haemodynamic data of patients in*

1	2	3	4	5	6	7
26	22	Recumbent	86	5.1	59	243
		Sitting	86	4.1	48	238
		Sitting	92	4.5	49	284
28	24	Recumbent	57	4.3	75	198
		Sitting	56	3.8	67	202
		Sitting	57	3.9	69	202
29	[illegible]	Recumbent	77	5.8	76	229
		Sitting	77	5.3	69	241
		Sitting	70	4.7	67	235
30	32	Recumbent	54	5.4	99	238
		Sitting	53	4.6	86	252
		Sitting	52	4.4	84	253
31	32	Sitting	68	4.4	65	249
32	25	Recumbent	86	5.7	66	227
		Sitting	86	4.5	52	242
		Sitting	87	5.7	66	254
33	16	Recumbent	64	5.4	85	238
		Sitting	66	4.7	71	254
		Sitting	64	4.2	66	235
34	23	Recumbent	63	5.5	88	213
		Sitting	73	4.8	66	227
		Sitting	76	4.6	60	216
35	21	Recumbent	51	4.8	94	207
		Sitting	51	4.8	94	215
		Sitting	54	4.4	82	215
36( )	26	Sitting(2)	128	3.0	23	[illegible]
		Sitting(2)	121	3.0	25	270
39	23	Recumbent	82	4.3	59	208
		Sitting	80	4.6	58	250
		Sitting	83	5.0	60	228
40	25	Recumbent	83	4.0	48	[illegible]
		Sitting	84	3.7	44	239
		Sitting	80	3.8	47	229

(1) Female
(2) Atrial fibrillation.

subacute myocardial infarction.

8	9	10	11	12	13	14
48	112	60	84	17	5.8	67
57	105	54	76	18	4.3	50
63	119	64	83	18	5.1	55
46	135	70	98	23	5.8	100
54	138	71	96	26	5.0	88
51	140	74	101	26	5.4	95
39	132	80	102	18	8.1	106
45	132	79	99	19	7.2	93
50	127	76	97	21	6.2	89
44	106	60	76	14	5.5	102
55	104	60	78	17	4.8	89
58	108	60	80	18	4.7	91
56	113	73	87	20	5.2	77
40	118	72	93	16	7.2	83
54	112	65	86	19	5.3	61
44	110	62	85	15	6.6	76
44	119	69	90	17	6.6	104
54	112	68	87	19	5.4	84
56	109	69	86	21	4.9	77
38	124	64	83	15	6.3	99
47	116	62	80	17	5.2	72
47	112	63	78	17	4.9	64
43	121	65	86	18	5.6	110
45	115	63	85	18	5.5	109
48	116	63	84	19	5.1	94
80	187	105	145	49	5.9	45
91	201	114	148	50	6.0	50
43	105	61	81	17	5.3	65
34	110	63	82	18	4.2	65
46	107	62	80	16	5.4	65
52	128	69	91	25	4.9	59
65	129	68	89	24	4.5	53
61	128	64	86	23	4.4	55

Table 4 continued. *Clinical data at reinvestigation of 19 male patients with*

1	2	3	4	5	6	7	8	9	10
4	49	190	79	3	Yes	No	600/310	No	Normal
25	51	180	82	3	Yes	Yes	880/430	No	Pathological Q and T waves in aVF II III and CR_4
26	63	172	71	3	Yes	No	730/400	No	Pathological Q and T waves in aVF II and III
29	56	170	76	3	N	No	930/500	No	Pathological Q and T waves in aVF II and III
30	59	184	77	4	No	No	660/330	No	Pathological Q wave in aVL, CR_2 and CR_4
31	47	175	93	4	Yes	Yes	840/440	Yes	Pathological Q wave in CR_2 and CR_4
33	58	174	80	3	N	No	850/440	No	Pathological Q and T waves in aVF II and III
34	49	186	82	4	Yes	N	810/390	No	Pathological T wave in aVF and pathological Q wave in III
35	39	170	69	3	Yes	No	620/350	No	Pathological Q and T waves in aVF II and III
39	59	184	69	4	No	No	650/370	No	Pathological Q and T waves in aVF III CR_2 and CR_4

arrhythmia 5 to 13 months after myocardial infarction.

11	12	13	14	15	16
Normal	Rest	20	68	Yes	N
	200	26	80		
	400	28	91		
Normal	Rest	16	102	Yes	Yes
	200	20	130		
Pathological Ventricular premature beats	Rest	20	100	Yes	No
	200	22	120		
Pathological	Rest	20	72	No	N
	200	24	102		
	400	28	125		
Normal	Rest	12	60	No	Yes
	300	16	84		
	600	26	105		
	900	—	120		
Normal	Rest	16	85	Yes	Yes
	200	24	104		
	400	26	115		
	600	28	124		
Normal Ventricular premature beats	Rest	20	65	No	No
	200	22	85		
	400	24	102		
Normal Ventricular premature beats	Rest	20	80	Yes	No
	200	26	100		
	400	30	115		
Normal	Rest	18	60	N	No
	200	24	95		
	400	26	100		
	600	28	120		
Normal	Rest	12	77	No	No
	200	20	110		
	400	20—24	125		

Table 5 *Hemodynamic data at rest and during work on bicycle ergometer of 19 male*

Patient	Body position or work load	Heart rate beats/min	Cardiac output l/min	Stroke volume ml	Oxygen consumption ml/min	Arteriovenous oxygen difference ml/l
1	2	3	4	5	6	7
3	Recumbent	58	4.9	84	279	57
	Sitting	61	5.5	91	295	53
	200 kpm/min	94	7.8	81	892	116
7	Recumbent	66	5.1	77	256	50
	Sitting	63	4.0	64	245	61
	200 kpm/min	83	7.1	85	882	125
9	Recumbent	57	5.0	88	235	47
	Sitting	57	4.3	75	255	60
	200 kpm/min	90	7.2	80	780	108
	400 kpm/min	116	9.3	80	1106	119
12	Sitting	89	4.8	54	274	56
	Sitting	86	4.9	57	274	56
	200 kpm/min	115	8.1	70	893	110
17	Recumbent	71	6.3	88	232	37
	Sitting	82	5.2	63	241	47
	[illegible]00 kpm/min	142	7.6	54	791	104
18	Recumbent	74	4.5	61	215	48
	Sitting	74	4.3	58	219	51
	[illegible]00 kpm/min	109	6.9	64	806	116
19	Recumbent	81	5.5	68	270	49
	Sitting	80	4.8	60	254	53
	200 kpm/min	112	7.2	64	842	118
21	Recumbent	62	5.1	82	174	34
	Sitting	59	4.9	83	208	43
	200 kpm/min	86	7.5	87	700	94
3	Recumbent	60	5.4	90	215	40
	Sitting	64	4.6	72	247	54
	[illegible]00 kpm/min	96	7.7	80	725	93

patients with sinusrhythm 3—13 months after myocardial infarction

Brachial arterial pressure					
systolic mm Hg	diastolic mm Hg	mean mm Hg	Resistance lu	Left ventricular work kpm/min	Left ventricular stroke work pm
8	9	10	11	12	13
112	68	82	17	5.4	94
109	66	84	15	6.3	104
131	75	91	12	9.5	100
146	79	103	20	7.1	108
148	78	100	25	5.5	87
170	88	122	17	11.7	141
148	72	103	21	7.0	123
142	67	92	22	5.3	94
165	68	100	14	9.8	109
184	76	109	12	13.8	119
172	101	129	27	8.5	95
166	99	126	26	8.4	98
204	99	143	18	15.7	136
137	77	97	16	8.3	116
138	77	100	19	7.0	86
176	97	125	16	13.0	92
133	85	109	24	6.7	90
127	80	100	23	5.8	79
160	99	122	18	11.5	106
170	78	116	21	8.7	107
139	65	96	20	6.2	78
160	70	103	14	10.0	90
135	71	99	19	6.9	110
140	73	98	20	6.5	111
192	75	130	16	12.2	142
104	66	82	15	6.0	100
94	58	76	17	4.8	74
113	64	85	11	8.9	92

Table 5 continued. Haemodynamic data at rest and during work on bicycle ergometer of 19 male

1	2	3	4	5	6	7
24	Recumbent	60	6.7	112	262	39
	Sitting	61	4.8	79	253	53
	200 kpm/min	78	9.6	123	871	91
25	Recumbent	71	3.9	55	268	69
	Sitting	80	3 9	48	280	73
	Sitting	76	4 1	53	283	70
6	Recumbent	75	4 4	58	242	56
	Sitting	75	4.2	56	212	51
	Sitting	76	4.5	60	229	51
29	Recumbent	74	5.5	74	256	47
	Sitting	67	4.5	68	261	57
	200 kpm/min	106	8.3	78	892	108
30	Recumbent	64	7.3	113	276	38
	Sitting	76	5 4	71	294	54
	200 kpm/min	85	9 2	108	903	98
31	Recumbent	74	5.3	72	301	57
	Sitting	71	5.0	71	295	59
	200 kpm/min	91	8.7	95	904	104
33	Recumbent	65	4 4	68	232	53
	Sitting	67	4 1	61	244	60
	200 kpm/min	1 6(1)	8.0	76	799	100
34	Recumbent	63	5 4	85	246	46
	Sitting	66	4 4	67	240	54
	200 kpm/min	103	8 9	87	877	98
35	Recumbent	54	5.2	96	222	43
	Sitting	58	4.3	74	230	54
	200 kpm/min	85	8.0	95	870	108
39	Recumbent	74	4 9	66	243	30
	Sitting	75	4.2	56	251	60
	200 kpm/min	111	8.2	74	908	110

(1) Ventricular premature beats.

patients with sinusrhythm 3—13 months fter myocardial infarction.

8	9	10	11	12	13
125	66	90	13	8.3	137
126	68	91	19	5.9	98
159	80	106	11	13.8	177
118	75	93	24	4.9	70
119	76	90	23	4.7	59
122	79	97	24	5.3	70
122	67	92	21	5.4	73
122	64	88	21	5.0	67
132	65	92	20	5.7	75
130	77	110	20	8.2	111
134	80	105	23	6.5	97
170	86	120	15	13.5	127
117	71	90	12	8.9	138
119	72	91	17	6.7	88
146	79	104	11	13.0	133
135	79	100	19	7.2	94
138	83	100	20	6.8	97
154	77	107	12	12.6	138
136	87	105	24	6.3	97
135	85	105	26	5.8	87
152	95	112	14	12.2	116
131	79	100	19	7.3	116
125	80	96	22	5.8	87
137	76	101	11	12.3	120
123	73	96	19	6.8	125
120	69	90	21	5.3	91
149	74	103	13	11.2	133
111	68	85	18	5.6	76
110	63	81	19	4.6	62
135	65	95	12	10.6	96

Table 6 *General data of male control subjects*

Subject	Age years	Height cm	Weight kg	Occupation	Days with bedrest before investigation
1	2	3	4	5	6
1	50	187	86	Desk clerk	0
	50	179	76	Fire brigade officer	16
3	50	167	67	Packer	0
4	55	176	56	Mechanic	0
5	55	172	96	Warf labourer	0
6	56	180	79	Builder	5
7	57	177	74	Builder	0
8	58	161	73	Butcher	6
9	62	167	67	Mechanic	0
10	64	180	6[illegible]	Cleaner	7
11	64	165	71	Labourer	8
12	67	178	71	Pensioner	0

investigated in the recumbent

Remarks
7
Volunteer
Patient. Lumbago-ischias
Patient. Cirrhosis hepatis
Patient. Spermatocele
Volunteer
Patient. Small haematemesis without fall in blood haemoglobin
Patient. Hernia inguinalis
Patient. Suspecta arthritis rheumatoides
Patient. Myeloma
Patient. Hypertrophia prostatae. Resectio ventriculi 40 days previously. One year later paroxysmal atrial fibrillation and ST T-changes in ECG
Patient. Acute cholecystitis 10 days previously. Dyspnoea on exertion
Patient. Hypertrophia prostatae

Table 7. Haemodynamic data at rest recumbent of male control subjects
Age 50 to 67 mean 57 years

Subject	Heart rate beats/min	Cardiac output l/m	Stroke volume ml	Brachial arterial pressure			Resistance units	Left ventricular work kpm/min	Left ventricular stroke work gm
				systolic mm Hg	diastolic mm Hg	mean mm Hg			
1	2	3	4	5	6	7	8	9	10
1	72	6.6	92	131	77	96	15	8.6	120
2	78	6.8	88	124	73	92	13	8.4	110
	68	5.8	85	127	73	98	17	7.7	113
3	78	7.4	95	144	74	107	15	10.7	138
4	69	5.3	76	108	54	74	14	5.3	77
5	60	5.3	88	167	85	110	21	7.9	132
6	60	5.7	94	95	55	69	12	5.3	88
	59	5.4	91	95	58	72	13	5.3	89
7	72	5.8	80	157	86	117	20	9.2	127
8	88	5.5	63	149	[illegible]	100	18	7.5	86
	84	5.0	59	149	74	102	21	6.9	82
9	61	4.8	78	[illegible]	75	99	21	6.4	105
	66	4.7	71	133	69	98	21	6.3	93
10	59	3.5	94	137	63	94	17	7.1	120
11	58	4.6	79	140	58	91	20	5.7	98
12	66	6.6	100	177	78	[illegible]	19	11.1	168

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